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Recent Advances in Understanding Enteric Pathogenic *Escherichia coli*

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doi:10.1128/CMR.00022-13

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SUMMARY

Although *Escherichia coli* can be an innocuous resident of the gastrointestinal tract, it also has the pathogenic capacity to cause significant diarrheal and extraintestinal diseases. Pathogenic variants of *E. coli* (pathovars or pathotypes) cause much morbidity and mortality worldwide. Consequently, pathogenic *E. coli* is widely studied in humans, animals, food, and the environment. While there are many common features that these pathotypes employ to colonize the intestinal mucosa and cause disease, the course, onset, and complications vary significantly. Outbreaks are common in developed and developing countries, and they sometimes have fatal consequences. Many of these pathotypes are a major public health concern as they have low infectious doses and are transmitted through ubiquitous mediums, including food and water. The seriousness of pathogenic *E. coli* is exemplified by dedicated national and international surveillance programs that monitor and track outbreaks; unfortunately, this surveillance is often lacking in developing countries. While not all pathotypes carry the same public health profile, they all carry an enormous potential to cause disease and continue to present challenges to human health. This comprehensive review highlights recent advances in our understanding of the intestinal pathotypes of *E. coli*.

INTRODUCTION

Theodor Escherich first reported the isolation and characterization of slender short rods from infant stool, which he named *Bacterium coli commune*, in his 1885 publication (reprinted in English [1]). Although the organism was later described under multiple synonyms and iterations by other researchers, the name *Escherichia coli* was not fully recognized until 1954 (2). Over 125 years later, *E. coli* is known as a harmless commensal of the gastrointestinal tract in warm-blooded animals and is used as the colloquial laboratory workhorse. However, there is an alternate side to *E. coli* afforded through gene gain and loss that enable it to become a highly diverse and adapted pathogen. Pathogenic *E. coli* can cause a broad range of human diseases that span from the gastrointestinal tract to extraintestinal sites such as the urinary tract, bloodstream, and central nervous system (3, 4).

Diarrheal illness causes much mortality worldwide, particularly in children under the age of 5 (5) (Fig. 1) and particularly in countries in sub-Saharan Africa and South Asia, whose children suffer many diarrhea-related deaths. While there are many etiological agents responsible for diarrhea, pathogenic *E. coli* is a major contributor. Recent data from the Global Enteric Multi-Center Study (GEMS), one of the largest case-control studies aiming to understand the burden of pediatric diarrheal disease in sub-Saharan Africa and South Asia (6), illustrate that enterotoxigenic *E. coli* and *Shigella* are among two of the four main causative agents of moderate to severe diarrhea among children in these areas (7). In addition, increased fatality rates are associated with enteropathogenic *E. coli* and certain enterotoxigenic *E. coli* strains, thus underlining the significant role of pathogenic *E. coli* in the global health burden of diarrheal disease. The *E. coli* scientific and clinical communities have made great strides in understanding *E. coli* microbiology, pathogenesis, ecology, and interactions with its host. These advances are essential for novel approaches to vaccines and treatments that prevent some of the serious sequelae and complications associated with *E. coli*-induced diarrheal illness.

While various pathotypes contribute to diarrhea, the clinical symptoms and outcomes, site and mechanism of colonization, and disease can differ (Table 1), exemplifying the diversity of *E. coli*. In this review, we focus on advancements in our understanding of enteric pathogenic *E. coli* since the comprehensive 1998 review by James Nataro and James Kaper (8). We discuss the six major diarrheagenic *E. coli* pathotypes: enteropathogenic *E. coli* (EPEC), Shiga toxin-producing *E. coli* (STEC) (e.g., enterohemorrhagic *E. coli* [EHEC]), *Shigella*/enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC), and enterotoxigenic *E. coli* (ETEC), as well as a new pathotype, adherent invasive *E. coli* (AIEC), in the context of detection, diagnosis, epidemiology, public health, pathogenesis, and human disease.

MICROBIOLOGY, ISOLATION, AND TYPING OF *E. COLI*

E. coli is a Gram-negative, oxidase-negative, rod-shaped bacterium from the family *Enterobacteriaceae*. It is able to grow both aerobically and anaerobically, preferably at 37°C, and can either be nonmotile or motile, with peritrichous flagella. *E. coli* is readily isolated from fecal samples by plating on selective media. The change in pH due to lactose fermentation can be used to differentiate between lactose-fermenting and non-lactose-fermenting strains, as lactose-positive *E. coli* colonies will appear red or pink on media such as MacConkey agar. Not all *E. coli* strains, partic-

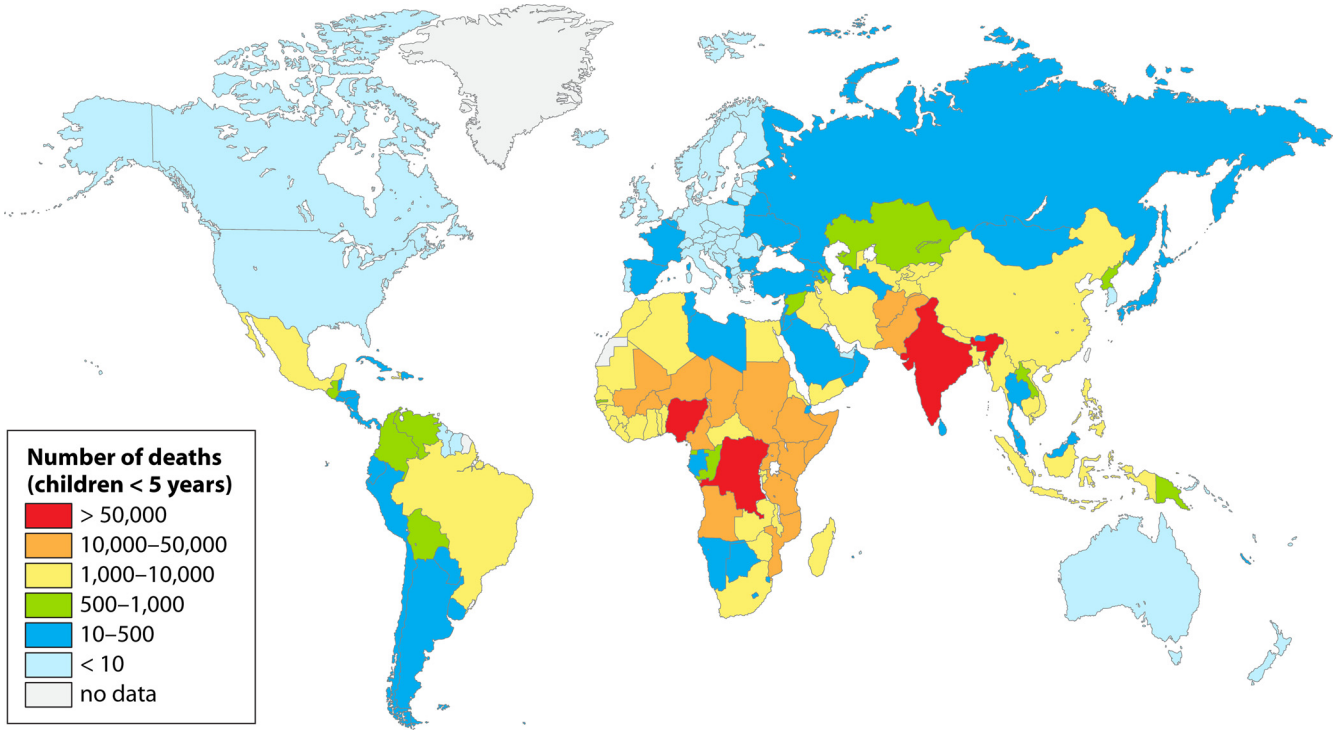

FIG 1 Global mortality from diarrhea in children under the age of 5 in 2010. Estimates of diarrhea-specific mortality among children under 5 for each country reflect high mortality in developing countries, with the highest tolls present in countries in sub-Saharan Africa and South Asia. Many etiological agents, including pathogenic *E. coli*, are responsible for diarrhea-related mortality in these children. Recent work published by GEMS found significant child mortality associated with EPEC and ETEC infections in developing countries (7). Source data for the map: World Health Organization (5).

ularly most EIEC and *Shigella* strains, ferment lactose, so caution must be used when using this diagnostic. While this selective plating can aid in isolating *E. coli* from Gram-positive bacteria and some other *Enterobacteriaceae* members, further morphological, phenotypic, and genotypic characteristics need to be tested for further identification and verification of pathotypes. Traditional culture techniques for pathogenic *E. coli* can be time-consuming and laborious. The adoption of molecular techniques (Fig. 2) has allowed for more rapid detection and identification of the different pathotypes. Current methods of identification of each pathotype are discussed in the appropriate sections below.

Classic serotyping is based on the Kauffman classification scheme, where the O (somatic) polysaccharides and H (flagellar) surface antigens are determined (8). Molecular methods such as PCR of genes involved in O-antigen biogenesis (e.g., *wzx* and *wzy* genes) and of *fliC* for the H antigen, can also be used to identify the serotype (9, 10). A designation of NM or H^- indicates an absence of the H antigen, and that the isolate is nonmotile. Currently, there are 174 *E. coli* O (10) and 53 *E. coli* H (9) antigens recognized; however only a small subset of O:H combinations are associated with disease.

While serotyping is informative for certain pathotypes (e.g., STEC O157:H7), it is not always useful for others due to isolates being untypeable or cross-reactivity between antigens. Other methods have been developed to type isolates for phylogenetic analysis, outbreaks, and surveillance investigations. Pulsed-field gel electrophoresis (PFGE) is considered the gold standard for typing and is applied in epidemiological investigations to discriminate between outbreak isolates (11). However, PFGE is laborious and time-consuming, requires technical expertise, and is not portable to laboratories that are not well equipped. An alternative method, called multilocus variable-number tandem repeat analysis (MVLA), has been shown to discriminate between sporadic isolates and outbreaks (12) and may be more portable than PFGE.

Multilocus sequence typing (MLST) has become a common method for typing pathogenic *E. coli* strains (13–18) and establishing their relatedness. A small number of housekeeping genes are sequenced and assigned a unique allele, and the allelic profile of the housekeeping genes can be used to give an isolate a sequence type (e.g., *E. coli* O104:H4 is ST678 and many STEC O157:H7 isolates are ST11, based on the MLST databases [see below]). Sequence types can be further grouped into clonal complexes based on their similarity. However, genetic diversity can be found within strains of a similar sequence type, so higher-resolution typing may be required to understand evolutionary relationships (19).

Currently three MLST schemes exist in publically curated databases: the EcMLST (http://www.shigatox.net), Institut Pasteur *Escherichia coli* MLST database (http://www.pasteur.fr/mlst), and MLST databases (20; http://www.mlst.net) enabling comparative results between laboratories. Recently, a public server that uses whole-genome sequencing to identify sequence types was set up (21; http://cge.cbs.dtu.dk/services/MLST). For more information on the use of MLST for molecular epidemiology, see a recent, thorough review dealing with this subject (22).

EVOLUTION OF PATHOGENIC *E. COLI*

As a population, *E. coli* strains can be assigned phylogenetically to 5 main groups, i.e., A, B1, B2, D, and E, with *Shigella* forming different groups (23) (Fig. 3). Commensal isolates mostly group in

TABLE 1 General overview of enteric *E. coli* pathotypes

Pathotype	Host(s)	Site of colonization	Disease(s)	Known reservoir(s)/ source(s) of contamination	Treatment	Adhesion[a]	Genetic identifiers
tEPEC	Children <5 yr, adults at high inocula	Small intestine	Profuse watery diarrhea	Humans	Oral rehydration, antibiotics for persistent cases	Attaching and effacing	*eae*$^+$, *bfp*$^+$, *stx*$^-$
aEPEC	Adults, children	Distal ileum, colon	Watery diarrhea, hemorrhagic colitis, HUS	Humans, animals, food, water	Hydration, supportive for HUS	Attaching and effacing[b]	*eae*$^+$, *stx*$^-$
STEC							*eae*$^{+/-}$, *stx*$^+$
EIEC/*Shigella*	Children <5 yr, adults, travelers, immunocompromised persons	Colon	Shigellosis/bacillary dysentery, potential HUS	Humans, animals, food, water	Oral rehydration, antibiotics	NA (invasive)	*ipaH*$^+$, *ial*$^+$, *stx*$^+$ (*S. dysenteriae*)
EAEC	Adults	Small intestine and/or colon	Traveler's diarrhea, HUS (*stx*$^+$)	Food, occasionally adult carriers	Antibiotics, oral rehydration	Stacked brick and/or invasive	*aatA*$^+$, *aaiC*$^+$ (535), other candidates (585)
	Children		Persistent diarrhea		Antibiotics, oral rehydration, potentially probiotics		
					Fluoroquinolones (685)		
ETEC	Immunocompromised persons		Persistent diarrhea	Food, water, humans, animals	Rehydration, antibiotics	CF mediated	CFs, LT, ST
	Children <5 yr, travelers	Small intestine	Watery diarrhea				
DAEC	Children (increasing in severity from 18 mo to 5 yr), adults	Intestine (uncharacterized location)	Persistent watery diarrhea in children, speculated to contribute to Crohn's disease in adults (809)	Unknown	Rehydration	Diffuse adherent and/or invasive	No uniform markers (802)
AIEC	Adults, children	Small intestine	Crohn's disease	Unknown	Antibiotics, surgical resection	NA (invasive)	Uncharacterized

[a] NA, not applicable.
[b] Only for LEE-positive STEC, not for LEE-negative STEC.

phylogroup A; however, not all *E. coli* pathotypes group together. For example ETEC was found to group with phylogenetic groups A and B1 (24), while EAEC grouped in phylogenetic groups A, B1, B2, and D (16), demonstrating the disparate nature of pathogenic *E. coli* genotypes. The population genetics and evolution of commensal and pathogenic *E. coli* strains have been recently reviewed (25).

Genome sizes of *E. coli* can differ by a million base pairs between commensals and pathogenic variants, and this extra genetic content can contain virulence and fitness genes. Comparative genomics have shown that *E. coli* genomes are split between a shared, conserved set of genes, called the core genome, and a flexible gene pool. A recent comparison of 186 *E. coli* genomes found approximately 1,700 homolog gene clusters shared in all genomes and a pangenome of about 16,400 gene clusters (26). The pathogenic ability of *E. coli* is therefore largely afforded by the flexible gene pool through the gain and loss of genetic material at a number of hot spots throughout the genome (23) (Fig. 4). For example, nearly one-quarter of the EAEC strain 042 genomic content is made up of genomic islands (27), similar to the percentage of unique genomic islands found in STEC O157:H7 strain EDL933 (28). DNA can be moved between prokaryotic hosts through mechanisms such as conjugation, transformation, and transduction (reviewed in reference 29), encoded by mobile genetic elements, resulting in horizontal gene transfer (HGT). Mobile genetic elements, such as transposons, insertion sequences, bacteriophages, and plasmids, can exist either integrated into the chromosome or through self-replication within the new host to provide new traits and fitness advantages. Most definable virulence factors found in pathogenic *E. coli* are derived from genetic mobile elements. For example, most of the genes for toxins and colonization factors (CFs) required for the pathogenesis of ETEC are found almost exclusively on plasmids (30), while EPEC and some STEC strains share a 35-kb cluster of virulence genes on a chromosomal pathogenicity island (PAI) called the locus of enterocyte effacement (LEE), which is required for the attaching and effacing (A/E) phenotype that is necessary for virulence (31). It should be noted that genes encoding products referred to as virulence factors in humans (e.g., LEE or Shiga toxins) may in fact contribute to survival in the environment or commensalism in other hosts and may provide a driving adaptive factor for retention of certain traits (32).

Bacteriophages play a large part in the genome plasticity of *E. coli*. Although many of these phages are seemingly defective, some still form infectious particles. A study of 18 phages found in an STEC O157:H7 isolate determined that some of these phages, including two phages that carry the Shiga toxin (Stx), can infect other *E. coli* strains, thus contributing further to HGT (33). While a large number of virulence factors can be phage associated, accessory genes can also be acquired on phages. In STEC isolates, tRNA genes for rare codons were introduced that are frequently used by foreign genes (34, 35).

The acquisition of new genes through HGT provides bacteria with a variety of new traits; however, gene loss can also favor the fitness or adaptation of a pathogen in a particular niche. There is a higher rate of gene loss in *Shigella* than in other pathogenic *E. coli* strains, which may be due to its restricted host range and lifestyle (36). Interestingly, *Shigella* has anywhere from 447 to 978 pseudogenes and gene deletions throughout its genome, which is more than any other pathogenic *E. coli* strains that were included in the

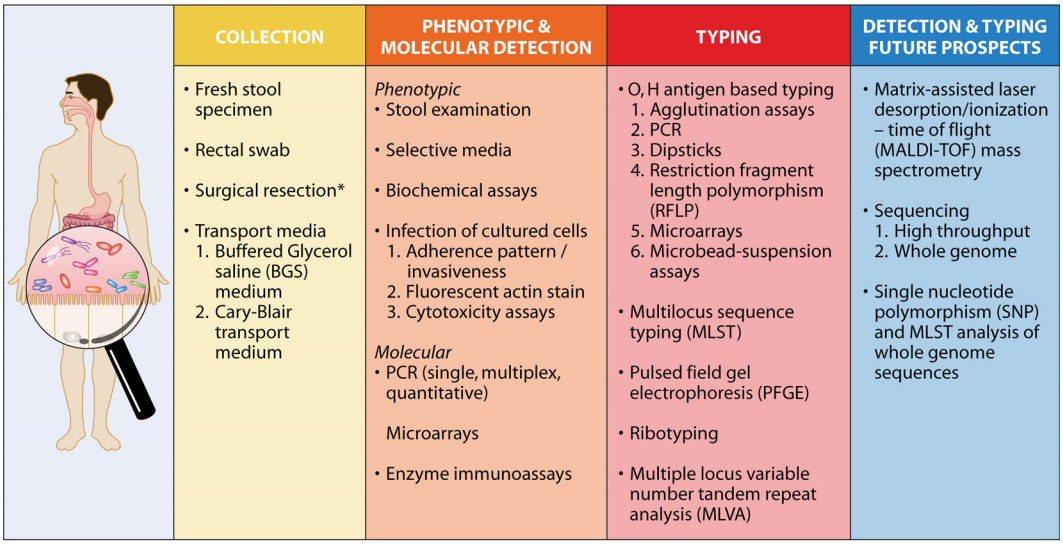

FIG 2 Diagnostic tools for intestinal pathogenic *E. coli*. *E. coli* causes a variety of diarrheal diseases in humans owing to specific colonization and virulence factors associated with each pathotype. As no single method can be used to detect and diagnose all pathogenic *E. coli* strains, a number of biochemical tests, typing methods, and molecular approaches have been developed to isolate *E. coli* from other enteric bacteria as well as to differentiate between particular pathotypes. Prospective methods such as whole-genome sequencing or high-throughput sequencing are becoming fast and affordable, providing much information about the pathogen that may be useful to clinicians, epidemiologists, and public health workers.

study (37). This loss of gene function has been described as pathoadaptivity, and it may be an underappreciated mechanism of pathogenesis that allows the survival of the pathogen in the host. Just a few examples of genes whose loss has helped *Shigella* adapt to an intracellular lifestyle are genes for lysine decarboxylase (LDC) (*cadA*), surface protease (*ompT*), and amino acid transport (*argT*) (reviewed in reference 38). Pathoadaptive events of EIEC and *Shigella* are discussed in more detail below. Pathoadaptation is not limited to *Shigella*, as loss of lysine decarboxylase activity has been shown in EPEC, ETEC, STEC, and EAEC (39, 40), and these pathoadaptive lesions may contribute to enhanced virulence in STEC O157:H7 and outbreak strains of EAEC (39, 41).

The description of pathotypes has usually relied on a signature set of genotypic and phenotypic traits. Because of the plasticity of the *E. coli* genome and increased sequencing and genomic studies, the designation of certain isolates into a pathotype becomes complicated. Prior to 2011, there were only been a few reports of Shiga toxin-producing EAEC causing bloody diarrhea and hemolytic uremic syndrome (HUS) (42, 43), but these are now more appreciated due to the *E. coli* O104:H4 outbreak in Germany (44). Characterization of isolates from this outbreak identified key virulence features belonging to different pathotypes, such as an aggregative adhesive phenotype *in vitro*, lack of the LEE PAI, and expression of a Shiga toxin (45). Therefore, these isolates can be considered a hybrid of both EAEC and EHEC (a subset of STEC), and it has been suggested that the STEC O104:H4 strain associated with the 2011 German outbreak be called enteroaggregative hemorrhagic *E. coli* (EAHEC) (46). Additionally, genome analysis of LEE-negative STEC has uncovered homologs and subunits of ETEC toxins in some isolates (47), further demonstrating the potential for the emergence of novel pathogenic *E. coli* hybrids.

As more genome sequences of pathogenic *E. coli* are being completed, it is clear that the genetic diversity of this organism is vast. As such, defining a pathotype of *E. coli* based on a small set of features is challenging, as many of these defining genes may not be restricted to a particular pathotype. However, it is still informative to consider the main pathotypes as a framework to an overview of enteric *E. coli*.

ENTEROPATHOGENIC *E. COLI*

Enteropathogenic *E. coli* (EPEC) belongs to a group of bacteria collectively known as attaching and effacing (A/E) pathogens based on their ability to form distinctive lesions on the surfaces of intestinal epithelial cells (IECs). Of the diarrheagenic *E. coli*, EPEC was the first pathotype to be identified. The term "EPEC" was first used in 1955 (48) to describe a number of *E. coli* strains epidemiologically related to a series of outbreaks of infantile diarrhea in the 1940s and 1950s (49, 50). Originally defined by serotype, EPEC strains are now classified based on pathogenic characteristics (8).

Classification

Atypical versus typical isolates. Most EPEC isolates correspond to conventional O serogroups (20, 51), but advances in molecular and cellular detection of EPEC have expanded the known repertoire of EPEC lineages to include strains that would not have been considered EPEC based on serotype alone (52). EPEC, unlike LEE-positive STEC, does not produce Shiga toxin and is distinguished from other diarrheagenic *E. coli* by the ability to form A/E lesions in the small intestine, a phenotype afforded to it by genes of the LEE (3). EPEC is further classified into "typical" and "atypical" subtypes based on the presence or absence of the *E. coli* adherence factor plasmid (pEAF) (53). Based on MLST, strains of EPEC fall into 4 clonal lineages, designated EPEC1 to EPEC4, that seem to have evolved through independent acquisition of the LEE and pEAF (15). Despite their formal designation as EPEC, typical and atypical isolates constitute two distinct groups of organisms, with some atypical EPEC (aEPEC) strains being more closely related to LEE-positive STEC in serotypes, genetic characteristics, virulence

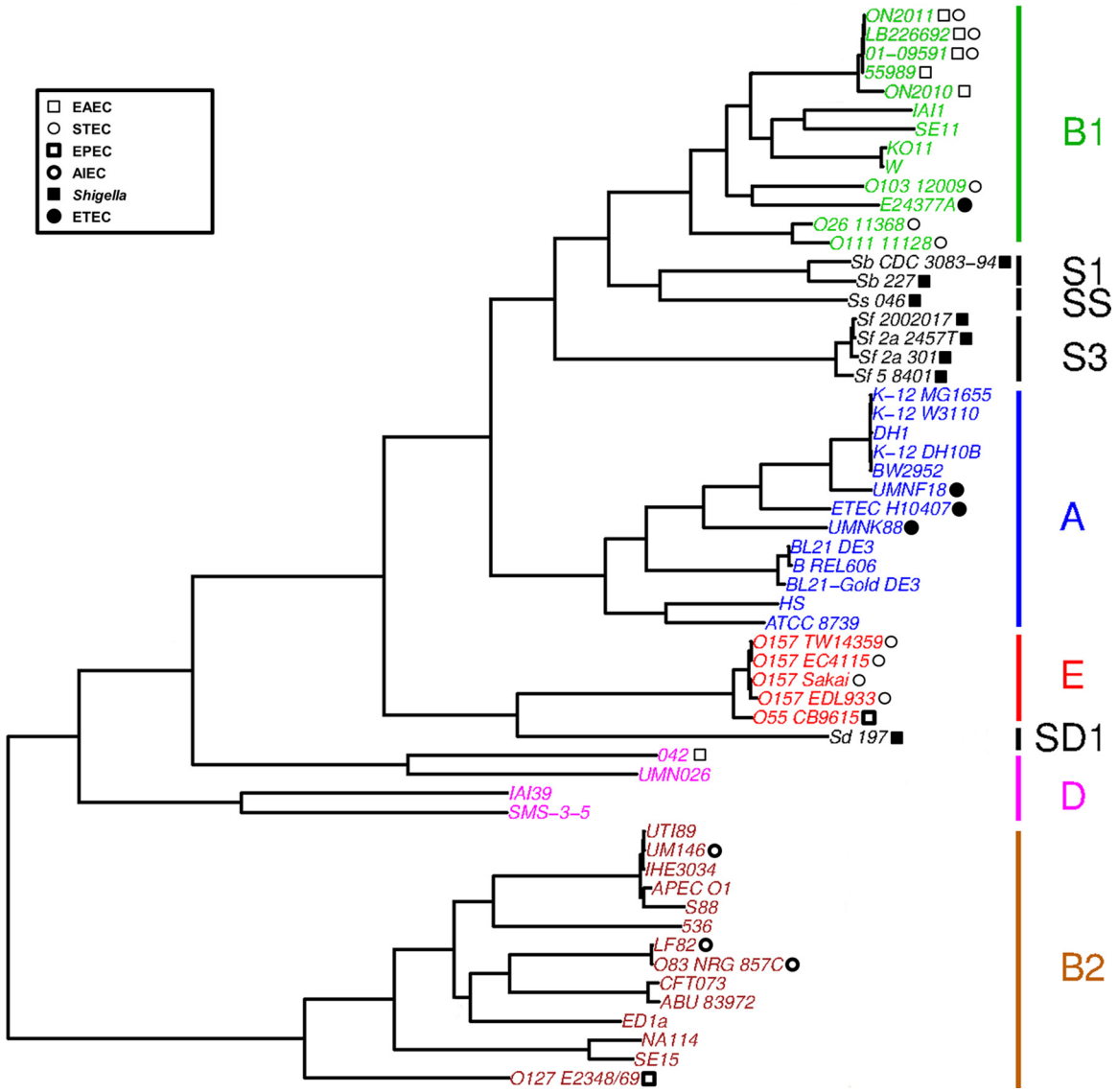

FIG 3 Phylogenetic tree of intestinal pathogenic *E. coli*. *E. coli* strains can be grouped into 5 main phylogenetic groups: A (blue), B1 (green), B2 (brown), D (pink), and E (red). *Shigella*/EIEC also form additional phylogroups (black). Pathotypes do not always group together in the same phylogroup. The hybrid EAEC and STEC strains are denoted with both an open square and open circle. Unmarked strains are either commensal, extraintestinal pathogenic *E. coli* (ExPEC), or avian-pathogenic *E. coli* (APEC). ETEC strains are isolated from both humans and animals, while DAEC is not represented in the phylogenetic tree. (Adapted from reference 19, which was published under a Creative Commons license.)

properties, and reservoirs (53). In contrast to typical EPEC (tEPEC), aEPEC is a highly heterogenous group, and despite its relatively high prevalence in asymptomatic children, aEPEC is now thought to be important in endemic diarrhea in children as well as in outbreaks (54). The highly pathogenic STEC serotype O157:H7 is closely related to aEPEC O55:H7 (55, 56), and recent comparative genomic and proteomic analyses indicate that STEC O157:H7 evolved from an O55:H7 ancestor, with an approximate divergence time of 400 years (57). Among aEPEC, strains of the O51 serogroup are the most frequently isolated, followed by O145, O26, O55, O111, and O119; however, many aEPEC strains are O/H-antigen nontypeable (58). Additionally, some aEPEC lineages have evolved from typical strains that have lost plasmid-carried virulence genes (59, 60), rendering lineage designations based on serotype alone inaccurate. Table 2 shows frequently isolated aEPEC and tEPEC serotypes, including common motile and nonmotile strains.

Epidemiology

Incidence. The epidemiology of EPEC infection has shifted since these strains were first identified in the 1940s and 1950s. Initially, EPEC was an important cause of infantile diarrhea in the developed world, but over the years it became much more prevalent in developing countries (8). The prevalence of EPEC infection varies between epidemiological studies based on differences in study populations, age distributions, and methods used for detection and diagnosis (61). In addition, geographic region and socioeconomic class may also contribute to the epidemiology of EPEC-

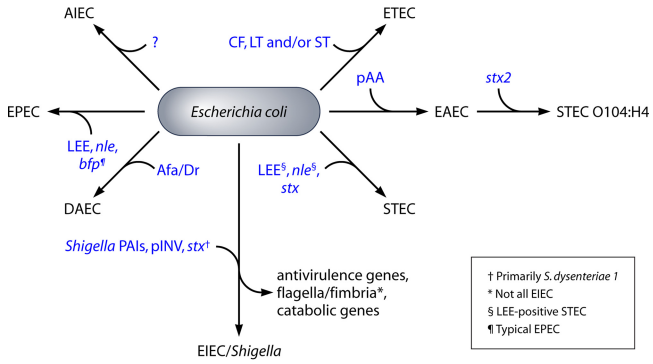

FIG 4 General overview of pathogenic gene acquisition and loss for different pathotypes. Gene gain and loss afford pathogenic traits to *E. coli* and ultimately lead to the pathotypes discussed in this review. Acquisition of genes is generally from mobile elements such as transposons, prophages, and plasmids. Typical EPEC carries the LEE and bundle-forming pilus gene (*bfp*), while most LEE-positive STEC strains (such as EHEC) also carry the LEE as well as Shiga toxin genes (stx_1, stx_2, or a combination). ETEC isolates carry enterotoxins LT and ST solely or together on plasmids, as well as colonization factors (CFs). Some DAEC isolates have acquired fimbriae that enhance adherence, called the Afa/Dr, while many virulence determinants for EAEC for some isolates are found on the pAA plasmid. Additionally, the O104:H4 serotype of EAEC, which was involved in the recent outbreak in Germany, acquired the stx_2 gene. EIEC/*Shigella* gained the ability to invade cells mainly through the pINV plasmid and acquired additional virulence traits in the form of chromosomal pathogenicity islands (PAIs). Subsequent pathoadaptation, including loss of antivirulence factors and motility, potentiate its virulence. Genes involved in the pathogenesis of AIEC are unclear. For more details about these genetic determinants, see the text.

induced diarrheal disease (62). In order to understand these issues, large case studies like GEMS are needed to more accurately assess the etiology and population-based burden of EPEC-induced diarrheal disease. Based on recent GEMS data, tEPEC was significantly associated with moderate to severe diarrhea in children under 2 years of age in Kenya, whereas aEPEC was not associated with this type of diarrhea at any of the GEMS sites (7). Overall, tEPEC was not strongly associated with cases of moderate to severe diarrhea, but when present, it was associated with an increased risk of death in patients aged 0 to 11 months (7). Recent estimates from the Centers for Disease Control and Prevention (CDC) on food-related illness in the United States listed only 4 hospitalizations as a result of EPEC infection (63); however, this pathogen continues to persist in other parts of the world and continues to be regarded as a serious threat to children under the age of 2.

The occurrence of diarrhea due to tEPEC decreases with age, and infections in adults are rarely reported. This apparent resistance in adults and older children has been attributed to the loss of specific EPEC receptors with age or development of immunity (8). For many years, infections with aEPEC were thought to predominate in industrialized nations while being relatively rare in the developing world (53, 64); however, recent data indicate that infections with aEPEC exceed those with tEPEC in both developing and developed countries (58, 61). For example, a 5-year study of children under 12 years of age in Thailand found that 71.8% of EPEC isolates were atypical in nature (65). Likewise, 92% of EPEC isolates collected from children in Brazil between 2001 and 2002 were atypical (66), compared to 38% in a 1998-1999 study (52). More recently, 39.3% of EPEC strains isolated from children with diarrhea in Iran tested positive for the *bfp* gene, while the remaining 61.7% appeared as aEPEC, which lacks *bfp* (67). Studies from Norway (68) and Australia (69) also suggest that aEPEC isolates are more commonly found among persistent cases of diarrhea than typical isolates. However, this notion cannot be generalized, as other studies still report tEPEC being more prevalent than aEPEC as a cause of diarrhea (70). Although many countries no longer consider tEPEC strains to be an important cause of acute diarrhea, the occurrence of severe disease outcomes associated with these infections has reemerged.

Transmission and reservoirs. As with other diarrheagenic *E. coli*, EPEC is transmitted from host to host via the fecal-oral route through contaminated surfaces, weaning fluids, and human carriers (71) (Fig. 5). Although rare, outbreaks among adults seem to occur through ingestion of contaminated food and water; however, no specific environmental reservoir has been identified as the most likely source of infection (8). The infectious dose in adult volunteers is high, at 10^8 to 10^{10} organisms (72, 73), while the actual dose required to cause disease during natural infection is unknown. EPEC outbreaks have been reported to show a seasonal distribution with peaks during the warm months (64, 68, 74, 75). In Norwegian children, 47.7% of EPEC isolates were identified between July and September (68). Humans are the only known reservoir for tEPEC, with symptomatic and asymptomatic children and asymptomatic adults being the most likely source (71). In contrast, atypical strains have been isolated from human and animal sources, including dogs, rabbits, monkeys, and sheep (76, 77). Many human and animal EPEC species are clonally related (77), sharing many virulence properties. A recent survey of laboratory rabbits obtained from commercial vendors and presenting with acute diarrhea found a disease-associated strain of aEPEC in animal stools and a significant association between diarrheic animals and the presence of aEPEC (78). These data suggest interspecies transmission as a means for human infection with aEPEC and rabbits as a possible animal reservoir. Strategies to prevent transmission and spread of EPEC include proper hand washing and improvements in sanitary conditions and freshwater supplies, as well as instruction on food, domestic, and personal hygiene (62).

Pathogenesis

In general, EPEC is a noninvasive organism and does not produce heat-labile (LT) or heat-stable (ST) enterotoxins. EPEC belongs to a group of pathogenic bacteria capable of causing A/E lesions on the surface of the host's intestinal epithelium (Fig. 6). EPEC is the prototype organism for strains causing A/E histopathology; however, other human pathogens, including LEE-positive STEC and *Escherichia albertii*, as well as several animal pathogens, such as

TABLE 2 Classification of frequently isolated typical and atypical EPEC serotypes and common motility phenotypes

Group	Serotypes	
	Motile	Nonmotile
Typical	O86:H34, O114:H2, O127:H6, O127:H40, O142:H6, O142:H34	O55:H6, O55:NM, O111:NM, O111:H2, O119:H6
Atypical	O55:H34, O86:H8, O111:H25, O119:H2, O125ac:H6, O128ab:H2	O26:H11, O55:H7, O111:H8, O111:H9

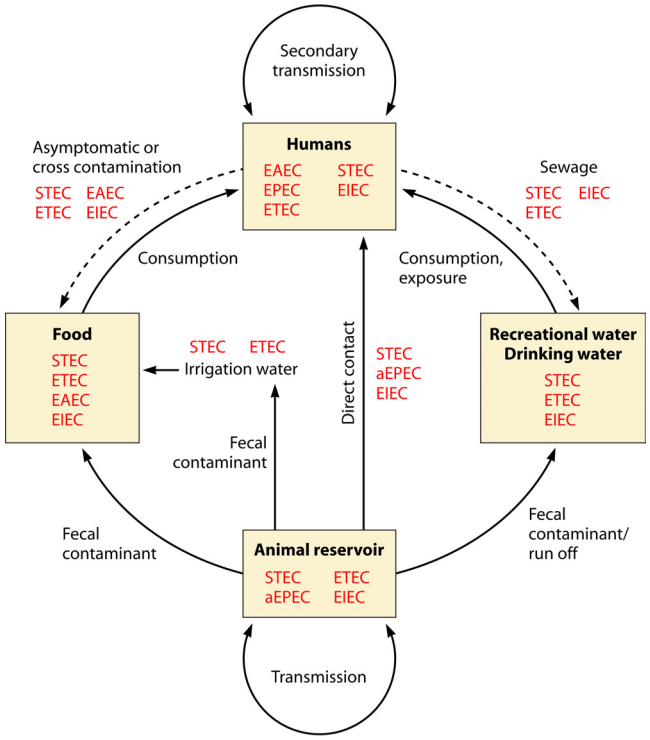

FIG 5 General overview of potential reservoirs and modes of transmission for pathogenic *E. coli*. Pathogenic *E. coli* strains can be found in various animal reservoirs and can spread between these and other animals. Fecal matter can contaminate food, irrigation water, or recreational/drinking water. Humans can become exposed following the ingestion of contaminated food or water or through direct contact with colonized animals. Secondary transmission can occur between humans, commonly in day care centers or nursing homes. Food can become contaminated through poor cooking practice, where, for example, uncooked meat could come in contact with other food. Additionally, symptomatic or asymptomatic food handlers can contaminate food, particularly when hand hygiene is inadequate. Contamination of recreational or drinking water can occur through exposure of human sewage.

rabbit-enteropathogenic *E. coli* (REPEC)/RDEC-1, porcine-enteropathogenic *E. coli* (PEPEC), dog-enteropathogenic *E. coli* (DEPEC), and *Citrobacter rodentium* (mouse), are also members of the A/E pathogen family (79). The A/E lesion is a hallmark of EPEC pathogenesis, characterized by effacement of brush border microvilli at the site of bacterial attachment (80). The dissolution of the intestinal brush border is accompanied by formation of actin pedestals that extend from the surface of the epithelium into the lumen (81). These pedestal-like structures are produced through secretion of a conserved bacterial receptor protein, Tir, via a type III secretion system (T3SS) that is absolutely required for EPEC pathogenesis (82). A three-stage model of EPEC pathogenesis was first described in the early 1990s by Donnenberg and Kaper, including localized adherence to host cells, signal transduction, and intimate attachment (83). Concomitant with intimate attachment, a series of bacterial effector proteins are injected into host cells, where they subvert actin dynamics and other host cellular processes (84).

Adherence. Initial attachment of tEPEC to the surface of the host intestinal epithelium is mediated by the bundle-forming pili (BFP) (85). BFP are type IV pili that tether individual bacteria to one another, producing a localized adherence (LA) pattern in the form of compact three-dimensional microcolonies that can be seen on HEp-2/HeLa cells within 3 h of infection (86). The LA phenotype correlates with EPEC1 and EPEC2 clonal-lineage strains carrying large EAF plasmids (87). The self-transmissible EAF plasmid pMAR2 is found among strains of the EPEC1 lineage and contains an intact transfer region, unlike pB171, which is more common among EPEC2 strains (88). Both plasmids share a pEAF backbone and, in addition to the *bfp* operon, carry a second virulence-related operon known as *perABC* (89). Between pMAR2 and pB171, the *bfp* and *per* loci share 99% sequence similarity (88), and both BFP and PerA have been shown to contribute to virulence in human volunteers (72). PerABC are plasmid-encoded regulators required for expression of the *bfp* and *perABC* operons as well as the LEE-encoded global regulator Ler (reviewed in reference 90), thereby linking BFP expression to expression of the LEE in tEPEC strains. In contrast, aEPEC strains do not harbor pEAF and thus do not produce BFP, resulting in the formation of loose clusters of bacteria on tissue culture cells. This pattern, known as "localized adherence-like" (LAL) (91), is slower to establish and can take up to 6 h to form (58). LAL is the most common pattern seen among aEPEC strains; however, some strains display alternate adherence phenotypes such as diffuse adherence (DA) and aggregative adherence (AA) (53) (Fig. 7). In addition to BFP, tEPEC strains encode a large surface protein, lymphocyte inhibitory factor (LifA), that contributes to epithelial cell adherence *in vitro* (92) and is required for intestinal colonization of mice by the related A/E pathogen *C. rodentium* (93). LifA has recently been identified as the largest secreted effector for any pathogen possessing a T3SS (94), and the gene encoding LifA has been found in the genomes of several A/E pathogens, including many isolates of aEPEC (92). The *lifA* gene is more commonly found among tEPEC rather than aEPEC strains (95); however, aEPEC strains harboring *lifA* have a significant association with diarrhea in children under 5 years of age (96). The *E. coli* common pilus (ECP) is an additional adherence factor, which is involved in epithelial cell colonization of commensal and pathogenic *E. coli* strains (97). In EPEC, ECP has been shown to act as an accessory adherence factor, playing a role during cell adherence and/or in bacterium-bacterium interactions (98). Although mutation of pilus genes in STEC O157:H7 leads to a substantially reduced ability to adhere to epithelial cells, the significance of ECP to EPEC pathogenesis has not been determined (97). The T3SS filament EspA has also been shown to play a role in initial brush border attachment. While BFP has been shown to be the predominant factor required for initial attachment of tEPEC (99), EspA filaments do promote attachment, albeit in a less efficient manner, and could mediate adherence of strains lacking BFP.

Signal transduction and intimate attachment. Formation of the A/E lesion occurs by subversion of actin dynamics within host cells and is mediated by the interaction between intimin and the bacterial translocated intimin receptor, Tir (100–102). Intimin is a 94-kDa protein encoded by the *eae* gene, which is found in all strains capable of inducing A/E histopathology (8). The N terminus of intimin is highly conserved among A/E pathogens, whereas the C terminus shows much less homology (103). Differences in the C-terminal region of intimin have been used as a basis for classification into several distinct subtypes, with the α and β subtypes more commonly found among tEPEC strains and the α, β, γ, ζ, δ, and ε subtypes being found among aEPEC strains worldwide (104). Intimate attachment of EPEC to intestinal cells in-

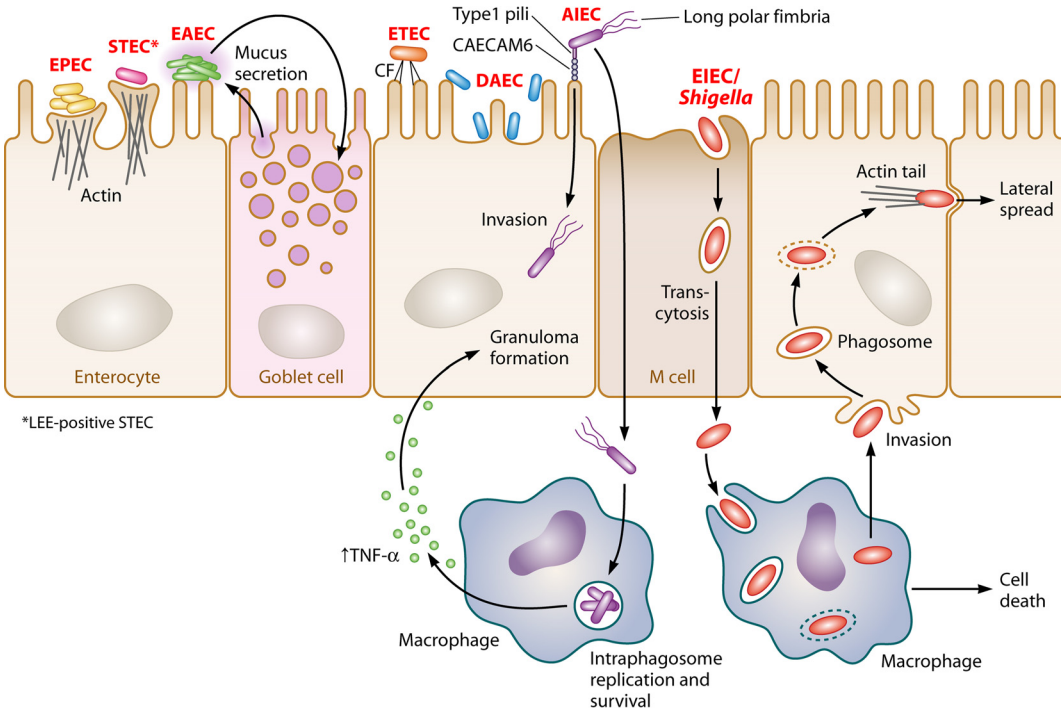

FIG 6 Adherence patterns of enteric *E. coli*. Pathogenic *E. coli* requires adherence to the host epithelium. Enteropathogenic *E. coli* (EPEC) (represented in yellow) and LEE-positive Shiga toxin-producing *E.coli* (STEC) (represented in pink) are extracellular pathogens that attach to the intestinal epithelium and efface microvilli, forming characteristic A/E lesions. Due to the presence of bundle-forming pili, EPEC is capable of forming microcolonies, resulting in a localized adherence (LA) pattern. Enterotoxigenic *E. coli* (ETEC) (represented in orange) uses colonization factors (CFs) for attachment to host intestinal cells. Enteroaggregative *E. coli* (EAEC) (represented in green) forms biofilms on the intestinal mucosa, and bacteria adhere to each other as well as to the cell surface to form an aggregative adherence pattern (AA) known as "stacked brick." Diffusely adherent *E. coli* (DAEC) (represented in blue) is dispersed over the surfaces of intestinal cells, resulting in a diffuse adherence (DA) pattern. Adherent invasive *E. coli* (AIEC) (represented in purple) colonizes the intestinal mucosae of patients with Crohn's disease and is capable of invading epithelial cells as well as replicating within macrophages. AIEC uses type I pili to adhere to intestinal cells and long polar fimbriae that contribute to invasion. Enteroinvasive *E. coli* (EIEC)/*Shigella* (represented in red) are intracellular pathogens that penetrate the intestinal epithelium through M cells to gain access to the submucosa. EIEC/*Shigella* escape submucosal macrophages by induction of macrophage cell death followed by basolateral invasion of colonocytes and lateral spread.

duces diverse signal transduction pathways within the host, leading to subversion of many cellular processes for the benefit of the pathogen. The genes required for signal transduction and production of the distinctive A/E lesion are carried on the conserved 35-kb LEE PAI that is found in all A/E pathogens (31, 105). The core LEE encodes a T3SS, the main function of which is to secrete protein components of the translocon (EspA, EspB, and EspD) and to drive effectors directly into host cells. There is a high degree of conservation in the genes encoding the T3SS apparatus itself, whereas the genes encoding effector proteins show considerable variation (106). In addition to the T3SS apparatus, the LEE carries regulatory genes and genes for secreted effectors and their related chaperones (107–109). In some tEPEC, aEPEC, and STEC strains, the presence of additional genes in the LEE flanking regions can extend the LEE up to 110 kb (106), although the role of these additional genes in pathogenesis is variable or unknown.

Secreted proteins. The EPEC genome contains seven LEE-encoded effector genes in addition to several non-LEE (Nle)-encoded effector genes, all of which exploit the LEE T3SS for delivery into host cells (107, 109, 110). Of the seven LEE-encoded effectors, one of the best studied is the translocated receptor, Tir. Tir is found at the tip of the pedestal, where it acts as a cellular receptor for the bacterial transmembrane protein intimin (102). In addition to its receptor function, Tir is also involved in effacement of microvilli and recruitment of cytoskeletal proteins for pedestal formation (79, 84). Recently, Tir has also been shown to inhibit NF-κB activity through tumor necrosis factor alpha (TNF-α) receptor-associated factors (111). The remaining six LEE-encoded effectors, Map, EspF, EspG, EspZ, EspH, and EspB, all have physiological roles relevant to A/E pathogen infection. Map stimulates formation of membrane filopodia and epithelial barrier disruption as well as mitochondrial dysfunction (112). Multifunctional properties have also been reported for EspF and EspG, both of which affect aquaporin localization, leading to diarrhea (113). Like Map, EspF localizes to mitochondria (114) and has been shown to disrupt tight junctions (115), while EspG alters host cytoskeletal components through its interaction with tubulin (116). EspZ promotes host cell survival (117), whereas EspH affects filopodium formation, participates in actin signaling during pedestal formation (118), and acts as a RhoGEF inhibitor (119). Both EspH and EspB are capable of inhibiting phagocytosis of EPEC by macrophages (120, 121). There is considerable variation in the number and type of Nle-encoded effectors among A/E pathogens (84). EPEC E2348/69 encodes at least 23 Nle effectors (94, 109), many of which are involved in dampening the host immune response. NleB, NleC, NleD, NleE, and NleH have all been shown to inhibit NF-κB activation through a variety of different mechanisms (122–129). In addition to immunomodula-

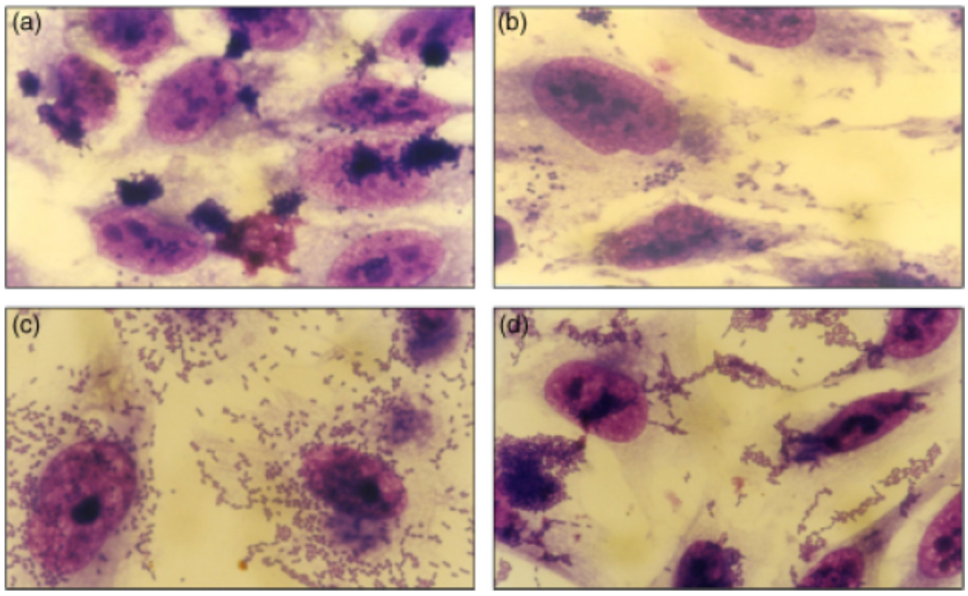

FIG 7 Adherence patterns of enteropathogenic *E. coli* (EPEC) on tissue culture cells. (a) Localized adherence (LA); (b) localized adherence like (LAL); (c) diffuse adherence (DA); (d) aggregative adherence (AA). (Reprinted from reference 58 with permission [© 2009 Federation of European Microbiological Societies].)

tory functions, Nle effectors such as EspJ have antiphagocytic properties (130), while NleA alters host protein secretion (131) and tight junction integrity (132) and inhibits vesicle trafficking (133). NleH is capable of modulating apoptotic response (134). For a thorough review of EPEC effector functions and host cellular targets, see references 84 and 135).

In addition to T3S effectors, some EPEC strains also encode the type V secreted virulence-associated protein EspC. EspC is a Per-activated serine protease autotransporter that acts as an enterotoxin, causing cytopathic effects on tissue culture cells (136) and rat jejunal segments (137). Cytotoxicity of EspC depends on a conserved serine protease motif as well as on epithelial cell internalization (138, 139). EspC has been shown to enter intestinal epithelial cells through a cooperative mechanism involving both the type V secretion system (T5SS) and the T3SS (138). Upon delivery to the exterior of the cell via the T5SS, EspC interacts with EspA and is internalized during pedestal formation via the T3SS translocon (138, 139). EspC confers enhanced lysozyme resistance to EPEC (140), and purified EspC has been shown to interact with and degrade hemoglobin (141) and to hydrolyze other proteins such as pepsin, factor V, and spectrin (142). Additionally, oligomerization of EspC gives rise to rope-like structures that serve as a substratum for adherence and biofilm formation as well as to protect bacteria from antimicrobial compounds (143). While the role of EspC in EPEC pathogenesis *in vivo* remains unclear, it has been suggested that EspC plays a significant role in EPEC survival (141, 143).

Mechanism of diarrhea production. Unlike that induced by ETEC, EPEC-induced diarrhea is not mediated by toxin production. However, the enteroaggregative heat-stable toxin 1 (EAST1) gene, *astA*, has been found in aEPEC strains significantly associated with diarrhea in Brazil (144). The *astA* gene has also been found in aEPEC O39:NM, O111, and ONT:H45, which have been associated with outbreaks in the United States (145), Finland (146), and Japan (147), respectively. The EAST1-encoding gene has been detected in many diarrheagenic *E. coli* strains as well as in nonpathogenic commensal strains (148); however, whether or not *astA* is expressed in aEPEC and triggers diarrhea has yet to be determined (58). EPEC persists in the small bowel by resisting phagocytosis (149) and dampening the host immune response (150). The exact mechanism of diarrhea production is not fully understood and likely involves a combination of different mechanisms (79). The speed of diarrhea onset implies a secretory mechanism rather than malabsorption as a more likely cause of diarrhea. Nonetheless, the effacement of microvilli at the sites of bacterial attachment could lead to a decrease in absorptive surfaces, thereby contributing to diarrhea through interference with proper absorptive channels. A number of T3S effectors are known to impact various water and ion channels of the intestinal epithelia and are thought to contribute collectively to EPEC-induced diarrhea but the exact contribution of each mechanism is unknown. Tir, Map, EspF, and EspH inhibit the sodium–D-glucose transporter (SGLT1), which is responsible for fluid uptake from the intestine (84, 151), while EspF and EspG alter localization of aquaporins, which also play a role in water transport (113). EspG and EspG2 are also responsible for disruption of chloride transport across the apical membrane resulting in decreased Cl^-/OH^- exchange activity (152). Additionally, disruption of tight junctions by EspF (153), EspG (154), and Map (155) leads to increased intestinal permeability, which could also contribute to EPEC-induced diarrhea.

Clinical Considerations

Symptoms. EPEC is a significant cause of infectious diarrhea that is often accompanied by fever, vomiting, and dehydration in children under 2 years of age (3, 8, 79). In human volunteers, the onset of diarrhea due to EPEC is fairly rapid, occurring as early as 2.9 h after ingestion of wild-type bacteria (156). Acute diarrhea is the most likely result of EPEC infection, but persistent cases, lasting more than 2 weeks, have also been reported (8, 71). In comparison

to infection with other diarrheal pathogens such as adenovirus, rotavirus, *Campylobacter*, and *Salmonella*, infection with EPEC is more likely to lead to development of persistent diarrhea and hospitalization (69). Other clinical features associated with EPEC-induced diarrhea include intolerance to cow's milk and an increase in failure to respond to oral rehydration therapy (61).

Detection. The traditional diagnosis of EPEC, involving O:H serotyping, has changed over the years as knowledge of this pathogen has increased. Most EPEC strains fall into well-established serogroups, but serotype designation is no longer a necessary trait for a strain to be considered "EPEC" (8). Diagnosis of EPEC is now made based on pathogenic characteristics that distinguish it from other *E. coli* species and subdivide EPEC into "typical" and "atypical" categories. EPEC is defined based on phenotypic properties, such as LA and A/E histopathology, that can be readily assessed using microscopy and cell culture techniques where tissue culture facilities are available, as well as by the presence or absence of genetic elements such as *eae*, *bfp*, and *stx* (8). The fluorescent actin staining (FAS) test, originally described by Knutton et al. (157), uses fluorescein isothiocyanate (FITC)- or rhodamine-conjugated phalloidin to label concentrated patches of filamentous actin beneath A/E bacteria on the surfaces of cultured epithelial cells. These concentrated spots of intense fluorescence are indicative of pedestal formation and signify a positive FAS test for A/E lesion formation (158, 159). In addition to the FAS test, a HEp-2 or HeLa cell adherence assay is another phenotypic test that can be used to distinguish tEPEC from aEPEC and other diarrheagenic *E. coli* such as DAEC and EAEC (86, 160, 161). Many clinical laboratories, however, do not have tissue culture facilities, and thus neither the HEp-2 adherence assay nor the FAS test can be routinely used for diagnosis in these settings. For this reason, genotypic tests for detection of EPEC are the preferred method of identification and generally involve DNA probe hybridization or PCR-based screens targeting *eae*, *bfp*, and EAF sequences. Genotypic detection methods have been adopted by clinical and research laboratories for the identification of EPEC strains; however, general concerns with respect to allelic variability in the *eae* and *bfpA* genes has generated interest in refining tests to include new genetic targets. For example, differentiating typical from atypical strains can be difficult based on *bfpA* alone. Under the current definition, isolates containing deletions in essential genes of the *bfp* operon outside *bfpA* would be classified as tEPEC while phenotypically behaving as atypical strains. Furthermore, certain isolates that produce BFP do not react with the EAF probe (53), while others that react with the EAF probe do not form BFP (59). Additional genome sequence data will be required to identify new genetic targets that can be used to correctly distinguish between tEPEC and aEPEC as well as between strains that cause acute diarrhea and those that do not.

Treatment. In most cases, EPEC-induced diarrhea is self-limiting and can be effectively treated with oral rehydration therapy. Persistent infections may require the use of antimicrobials; however, the antibiotic resistance profile of EPEC is an important factor in determining the success of treatment in response to infection, as several clinical isolates exhibiting a high degree of resistance to standard antibiotics have been reported (162). In addition to resistance patterns, other factors limiting the effectiveness of antibiotics in the treatment of EPEC infections are cost and supply in developing countries, where EPEC infections are common (163).

Antibiotic resistance. The prevalence of antibiotic-resistant EPEC in developing and developed countries is increasing (162). Antibiotic-resistant EPEC has been found across many continents, with reported cases in the United States (164), the United Kingdom (165–167), Brazil (52), Iran (168), and Singapore (169). EPEC displays resistance to a range of antibiotics, including penicillins, cephalosporins, and aminoglycosides (162). A recent study of 149 EPEC strains isolated from children in Brazil found that resistance was more common among tEPEC strains than among aEPEC strains (52). In addition, markers for a conjugative multidrug resistance plasmid were detected in 30% of atypical isolates, compared to only 4% of typical strains (52).

Vaccines. The spread of infections due to EPEC has been well documented with numerous case studies in hospitals and nurseries (8, 71); however, no vaccines are currently available to control its spread. Antibodies against EPEC O antigens and outer membrane proteins have been found in breast milk (170, 171), and protection from mother to infant can be transmitted through colostrum IgA (172, 173). Antibodies from maternal colostrum and serum samples have been shown to recognize EPEC surface antigens such as Bfp and intimin, as well as the secreted proteins EspA and EspB, albeit with various degrees of reactivity that are likely dependent on antigen type and sample source (174). Therefore, several candidate vaccines based on conserved virulence proteins such as EspB (175, 176), BfpA (176, 177), and intimin (178) have been explored. Purified recombinant versions of EspB and BfpA were capable of eliciting an antibody response in rabbits and showed antigenic potential in humans when reacted with secretory IgA (sIgA) present in the stools of diarrheic pediatric patients (175), indicating that an immune response to these potential vaccine subunits can be produced at an early age (176). REPEC Δ*eae* mutants show reduced adherence to intestinal brush borders and protection from homologous rechallenge using a rabbit model of infection (178). Conversely, human volunteer studies have shown that 36% of individuals who ingested an EPEC Δ*eae* strain still developed diarrhea (156), indicating that live attenuated mutants of this nature may not be viable candidates for human vaccine development. In adult volunteers, diarrhea developed in 10% of individuals who consumed the Δ*espB* mutant, compared to 100% who consumed wild-type EPEC (179). Similarly, a Δ*bfp* mutant was significantly less virulent to human volunteers than wild-type EPEC but was still able to colonize and cause diarrhea in some cases (72). These data suggest that more than one antigenic factor may be required for successful vaccine development. Recently, bacterial ghosts devoid of cytoplasmic contents but expressing all EPEC surface components were constructed and used in vaccination challenge experiments with mice (180). Vaccinated mice showed 84 to 90% protection when challenged with wild-type EPEC, compared to no protection in control mice (180). Homologous rechallenge with wild-type EPEC resulted in a reduced severity of disease but had no effect on incidence of diarrhea (181).

SHIGA TOXIN-PRODUCING *E. COLI*

The presence of the Shiga toxin 1 or 2 gene (stx_1 or stx_2), typically acquired by a lambdoid bacteriophage, in an isolate of *E. coli* qualifies it as Shiga toxin-producing *E. coli* (STEC) or verocytotoxin-producing *E. coli* (VTEC). Despite there being over 400 STEC serotypes identified, only a subset of these have been correlated to illness in humans (182). STEC encompasses a diverse pathotype that can cause mild to bloody diarrhea and HUS.

TABLE 3 Common STEC serotypes discussed in this review[a]

O serogroup	H antigen(s)	*eae*	Seropathotype[b]	HC	HUS[c]
O26	NM, H11	+	B	+	+
O45	NM	+	B	+	−
O91	NM, H21	−	C	+	+
O103	NM, H2	+	B	+	+
O111	NM, H8	+	B	+	+
O113	H21	−	C	+	+
O121	H19	+	B	+	+
O145	NM, H25, H28	+	B	+	+
O157	NM,[d] H7	+	A	+	+

[a] HC, hemorrhagic colitis; HUS, hemolytic uremic syndrome.
[b] See reference 248.
[c] See references 185, 186, and 871.
[d] Sorbitol fermenting.

Enterohemorrhagic *E. coli* (EHEC) is a subset of STEC and was originally described by its association with hemorrhagic colitis (HC), which was clinically distinct from shigellosis, and had genotypic and phenotypic features that differed from those of EPEC (8, 183). Generally, EHEC is LEE positive and forms A/E lesions as does EPEC. However, the term EHEC has also been used in the literature to describe LEE-negative STEC strains, such as serotypes O91:H21, O104:H4, and O113:H21, that have caused HC and HUS. STEC O104:H4 can be considered a hybrid of EHEC and EAEC and is discussed both in this section and in the section on enteroaggregative *E. coli* below, as the genetic backbone is more closely related to that of an *stx*-negative EAEC O104:H4 isolate from Central Africa (44, 45). It has been suggested in the literature that this strain be called EAHEC (46).

The most common EHEC serogroup is O157:H7 and has been the subject of many studies, especially for molecular mechanisms of pathogenesis. While STEC O157:H7 has been classified as an adulterant in beef since 1994, the U.S. Department of Agriculture (USDA) has recently declared 6 more EHEC serogroups, i.e., O26, O45, O103, O111, O121, and O145 (also known as the "Big 6"), to be adulterants (184), as they are the most commonly found non-O157 STEC strains associated with severe illness in humans. It should be noted, however, that the prevalence of non-O157 STEC differs geographically (185).

There are important implications of STEC in veterinary microbiology (animals as reservoirs), environmental microbiology (land and water), food microbiology (contamination, handling, and preparation), and clinical microbiology (public health, surveillance, and human illness). The wealth and diversity of literature on STEC exemplify its impact on many fields of study.

Classification

Common serotypes. In most parts of the world, STEC O157:H7 is the most common serotype that causes human illness. However, it is becoming evident that non-O157 STEC strains also cause significant human illness (Table 3). A study of non-O157 STEC human illness in the United States between 1983 and 2002 found that the most common serogroups were the "Big 6" described above (186). In Australia, non-O157 STEC strains made up 42% of all typeable STEC isolates, with O111 and O26 being the most common serogroups (187). Likewise, these serotypes are common in many parts of the world, but the overall prevalence differs geographically (reviewed in reference 185). Sorbitol-fermenting O157:NM (SFO157:NM) was found to be isolated in approximately 17% of HUS cases from Germany and Austria between 1996 and 2006 (188).

Lineages. There are four different clonal lineages of STEC: EHEC 1, which includes O157:H7 and SFO157:NM; EHEC 2, which contains non-O157 STEC serotypes such as O111:H8 and O26:H11; STEC 1, which contains LEE-negative STEC serotypes such as O113:H21 and O91:H21; and STEC 2, which includes serotypes O45:H2 and O103:H2/H6 (55; http://www.shigatox.net). STEC O157:H7 strains have been further classified into 2 different lineages (lineages I and II), based on lineage-specific polymorphism assay 6 (LPSA-6) (189), and 9 different clades, based on single nucleotide polymorphism (SNP) analysis (190). The phylogeny of LEE-negative STEC strains is disparate, as they appear to be more related to other *E. coli* pathotypes than LEE-positive STEC strains and may have evolved on multiple occasions (47).

Epidemiology

The surveillance and control of STEC have become a major focus of public health authorities. While the primary focus was placed on the detection of *E. coli* O157:H7, it has become apparent that non-O157 STEC is also a major contributor to sporadic cases and outbreaks in North America, Australia, and Europe. A specific program called the Foodborne Diseases Active Surveillance Network (FoodNet) provides active surveillance of food-borne illness in the United States (191, 192). It should be noted that FoodNet provides surveillance for 15% of the population of the United States. Additionally, PulseNET was created to provide a database of standardized PFGE fingerprints, allowing for comparisons to aid in epidemiological investigations during outbreaks (11). Since its introduction, PulseNET networks have been set up internationally, except in sub-Saharan Africa.

Incidence. In the United States, there were 463 STEC O157:H7 (0.97 per 100,000 population) and 521 non-O157 STEC (1.10/100,000) cases reported by FoodNet in 2011 (193). Hospitalization rates due to these cases were over 2-fold higher for STEC O157:H7 (43.4%) than for non-O157 STEC (18%). Similarly, the case-fatality rate for O157:H7 was about 2-fold higher than that for non-O157 STEC. What is promising is the fact that the incidence of STEC O157:H7 has dropped 42% in 2011 compared to the incidence in 1996 to 1998 (193). Increased incidences of non-O157 STEC were identified in a statewide study in Washington between 2005 and 2010; the authors suggest that this increase may be due to changes in testing (194). The incidence in Canada, reported by the National Enteric Surveillance Program (NESP), has also improved for STEC O157:H7 cases in 2010 (1.18/100,000) compared to the incidences in 2005 (2.28/100,000) and 2006 (3.0/100,000) (195). NESP reported that the non-O157 STEC incidence has not changed in the last 10 years. In Australia, the overall incidence of STEC illness from 2000 to 2010 was reported to be 0.4/100,000, with a slight increase in incidence over this time (187). In Europe, the European Center for Disease Prevention and Control (ECDC) and European Food Safety Authority (EFSA) report on STEC incidences from 24 European Union member states; they reported that the overall incidence of STEC in 2009 for the European Union was 0.75/100,000. Ireland and Denmark had incidence rates significantly higher than those in other countries (5.33 and 2.90/100,000, respectively) (196). It should be noted that the ECDC/EFSA recommends not comparing incidences between countries due to differences in detection.

STEC is also prevalent in developing countries such as Argentina, which has been described in the literature to have the highest worldwide incidence of HUS in children under the age of 5 (197). This may be due to excessive exposure to known risk factors associated with STEC infections, including meat consumption, playing in recreational water, and poor personal hygiene (198). In contrast, neighboring Brazil has low incidences of HUS (199), and cases of STEC O157:H7 are uncommon (200). While STEC infections are identified in other developing countries (201, 202), overall surveillance and clinical diagnostics are lacking.

Transmission, reservoirs, and sources. Transmission of STEC is fecal-oral (Fig. 5), and the infectious dose is thought to be very low. For one STEC O157:H7 investigation, it was determined that the beef patty with the highest level of contaminated had only 675 organisms, and it was suggested that the infectious dose could be lower (203). Similarly, another study following an outbreak estimated a dose as small as one STEC O111:NM organism per 10 g of sausage (204). Low doses such as these were also estimated in sausage in the United States (205). These estimates of infectious dose may be further complicated by the ability of STEC to form viable-but-nonculturable (VBNC) cells when faced with stress in the environment. VBNC cells have been shown to form on food and are still able to produce the Shiga toxin (206); however, it is still unknown what impact VBNC cells have on human illness.

Ruminants such as cattle (both meat and dairy) are widely known to be major reservoirs for pathogenic STEC, and exposure to their fecal matter represents an important source of human illness (reviewed in reference 207). A recent survey of ground beef in the United States showed that approximately 24% of samples tested were positive for *stx* genes by PCR, with only a small proportion of isolates being potentially pathogenic to humans (208). STEC O157:H7 has been isolated from other animals and insects, which include but are not limited to swine, sheep, deer, wild boars, rabbits, birds, dogs, rodents, and insects (recently reviewed in reference 209). This demonstrates the capacity for human exposure to STEC or for further dissemination of STEC to other animals. Certain STEC serotypes, such as SFO157:NM, and O104:H4, have rarely been or have not been reported as being isolated from animals (210, 211).

Exposure by direct contact with animals or with their feces from petting zoos and farms is an important route of exposure of STEC. It is estimated that animal contact constitutes 8% of non-O157 and 6% of O157:H7 STEC illnesses in the United States (212). A study in Scotland showed that cattle feces can contain from 100 to over 10^6 CFU of STEC O157:H7 per gram of feces (213). Increased shedding was associated with STEC mucosal colonization at the rectoanal junction (213, 214). High-level shedding has implications for within-herd and between-farm transmission, as well as direct-contact and potential environmental exposure of humans, and has been discussed in more detail by Chase-Topping et al. (215).

In Scotland, it was estimated that 54% of STEC O157:H7 outbreaks were due to environmental exposure (216). STEC has been shown to survive in the soil for up to several months (217, 218), whereas other studies have shown survival for up to a year in nonaerated sheep manure (219) and for at least 3 weeks in various farm animal feces, including that of cattle and swine (220). For different water sources, survival of STEC O157:H7 for at least 2 months under laboratory conditions has been reported (221). The ability of *E. coli* to survive in the open environment is complex, as it must face many factors, such as temperature, nutrient availability, osmolarity, pH, moisture, and microbial communities (reviewed in references 222 and 223).

Contaminated food and water are responsible for many sporadic and outbreak-related illnesses due to STEC. In the United States, it is estimated that 68% of O157:H7 and 82% of non-O157 STEC illnesses are food related (224). Meat can become contaminated through contact with animal feces during slaughter and processing of colonized animals, whereas vegetables may become contaminated through the use of manure as fertilizer or through contaminated irrigation water (225). Contaminated runoff and irrigation water can also taint nearby water sources, affecting rivers, lakes, and private drinking water wells. Foods that have been involved in human illness include uncooked hamburger, sausage, raw milk and dairy products, apple cider, lettuce, spinach, and sprouts (226). Similar to its environmental survival, STEC has been shown to replicate and survive for long periods of time on various food sources (reviewed in reference 226). Washing of food may also become more difficult for certain foods, as both STEC O157:H7 and O26:H11/NM were shown to attach to spinach using the virulence factor EspA (described in the section on enteropathogenic *E. coli* above) (227), while Saldaña et al. reported internalization inside spinach leaf tissue (228). Lettuce that is damaged, from shredding or bruising, also provides an opportunity for STEC O157:H7 to replicate more rapidly than on intact leaf surfaces (229).

Nonsource exposure, such as person-to-person transmission, is thought to contribute to approximately 19% of cases during an STEC O157:H7 outbreak (230). Additionally, asymptomatic shedders may also be a source of person-to-person transmission, especially in the case of food handlers or when highly susceptible recipients are involved (231).

Outbreaks. Outbreaks have continued to be reported for O157:H7 and non-O157 STEC worldwide. Over 1,000 cases and 2 deaths were reported in New York in 1999 due to water well contamination by cattle manure runoff that contained STEC O157:H7 (232). The following year, STEC O157:H7 and *Campylobacter* contaminated a municipal water source in Ontario, Canada, where there were over 2,000 suspected illnesses (233). STEC O157:H7 has also been detected in outbreaks of bloody diarrhea in countries in sub-Saharan Africa, such as Cameroon (234) and the Republic of Congo (235). An STEC O111:NM strain was the source of illness for 341 people in Oklahoma in 2008, which was reported as being the largest O111 outbreak in the United States (236, 237). Norway was hit with a food-borne outbreak of a rare serotype, STEC O103:H25, that resulted in an abnormally high incidence of HUS (238). The largest reported U.S. STEC O26:H11 outbreak occurred in 2010 at a child care center, where shedding was detected for up to a month (239). SFO157:NM caused an outbreak in Germany in 2002 that had an 11% case-fatality rate and an outbreak in Scotland, in which 8 of 18 patients developed HUS (240, 241).

While these are only a few examples of outbreaks and their sources, many more occur annually worldwide, demonstrating the hazardous effect that STEC has on human health. The large STEC O104:H4 outbreak in Germany will be addressed in section on enteroaggregative *E. coli* below.

Pathogenesis

Despite the separation of LEE-positive STEC strains into distinct lineages (EHEC 1 and EHEC 2), acquisition of major virulence factors appears to have occurred in parallel in both lineages (51). Analysis of single nucleotide polymorphisms (SNPs) in STEC O157:H7 showed that they could be further divided into 9 clades (190), where more virulent isolates grouped together in clade 8. Although the majority of STEC pathogenesis studies have been done with serotype O157:H7 (EHEC 1 lineage), the shared virulence factors found in both EHEC lineages suggests that they are likely similar mechanistically. A/E lesion formation is caused by the T3SS encoded by the LEE, which injects small effector proteins into the host cell (3, 4), and thus are grouped as LEE-positive STEC. LEE-negative STEC strains have also been isolated in cases of HC and HUS, but since they lack the T3SS, colonization and pathogenesis are likely caused by a unique set of virulence factors.

Many STEC virulence factors are found on large virulence plasmids or genomic islands. Genomic islands unique to STEC O157:H7 strain EDL933 were designated O islands (OIs) (28), and this nomenclature has been used to describe islands in other strains. STEC O157:H7 strains carry a plasmid called pO157 that contains a catalase-peroxidase gene (*katP*) and genes for other virulence factors such as an enterohemolysin (*ehx*) and *toxB* and *espP* (242, 243). SFO157:NM strains carry a significantly larger virulence plasmid, pSFO157, which lacks *katP*, *toxB*, and *espP* but carries a locus for an adhesin, *sfp* (244). Plasmids in non-O157 STEC strains, such as serotypes O26, O103, O111, and O145 have gene contents similar to that of pO157, with some variances (35, 245, 246). Interestingly, an exceptionally large plasmid, pO26-Vir, from an O26:H11 isolate, also carries a cluster that encodes a type IV pilus (245).

In contrast to virulence plasmids found in LEE-positive STEC, pO113 from the LEE-negative STEC O113:H21 contains many different virulence factors, including a subtilase (*subAB*), mucinase (*epeA*), and an adhesin (*saa*), but carries *espP* and *ehx* (247).

Seropathotypes. A classification system was created to group STEC strains based on their association and frequency of detection with the incidence and severity of human illness (248). This system included 5 seropathotype classifications (A through E), with the strains most frequently causing severe illness falling in seropathotype A (exclusively STEC O157:H7 and SFO157:NM) and serotypes such as O26:H11, O103:H2, O111:NM, O121:H19, and O145:NM falling in seropathotype B. LEE-negative STEC strains, such as O91:H21 and O113:H21, were grouped in seropathotype C (Table 3). Virulence gene profiling in conjunction with seropathotype classifications has been used to assess the potential of STEC pathogenicity in humans (249, 250); however, a recent opinion published by the EFSA discussed limitations of seropathotype classifications, and proposed a new modified scheme called the HUS-associated serotype(s) (HAS) (251).

Virulence. With the variety of serotypes and virulence factors found among STEC strains, it is of little surprise that the severity of disease can differ. While stx_1-containing STEC can lead to HUS, the presence of stx_2 is associated with more severe human disease than that of stx_1 (252). In one study, stx_2 and a variant, stx_{2c}, were the only subtypes found from HUS cases (253). Recently, it has been noted that there has been an increase in stx_2-carrying STEC O26:H11/NM relative to stx_1 in Europe, which has been associated with higher rates of severe disease outcomes (254). Additionally, the presence of the LEE also correlated with disease severity (252). OI-122 has been correlated with severity of disease in non-O157 STEC, and in particular, certain *nle* genes carried by this island may be indicative of higher virulence (248, 255). Further analysis by Coombes et al. looked at the molecular risk associated with *nle* genes found on different OIs and demonstrated their prevalence in isolates that cause HUS (250).

Manning et al. contrasted a spinach outbreak and a lettuce outbreak of STEC O157:H7 from 2006 with the average of 350 STEC O157:H7 North American outbreaks and noted that the two outbreaks had much higher HUS rates (13% versus 4%) (190, 256). These more virulent isolates clustered in clade 8. The reasons for the increased virulence are unclear; however, these clade 8 isolates frequently carry both stx_2 and stx_{2c}, have a higher basal level of stx_2 expression, are more adherent to epithelial cells *in vitro*, and have higher expression of virulence factors (190, 257–259). A recent comparison of a clade 8 genome to that of the STEC O157:H7 outbreak strain from Sakai, Japan, identified putative genes that may contribute to higher pathogenicity (260).

Mechanisms of disease. Following ingestion, STEC must survive the low acidity of the stomach; like other *E. coli* strains, STEC strains have the ability to survive in low pH, which has been recently discussed by Hong et al. (261). In order to colonize the intestinal mucosa, STEC must attach to epithelial cells, which is achieved through a myriad of pili and fimbria (reviewed in reference 262). Below we discuss these mechanisms of pathogenesis depending on LEE status, including colonization factors and the contribution of other virulence factors to disease.

(i) Shiga toxin. Shiga toxin is the main characteristic that defines STEC and is the key virulence factor in STEC causing HUS. Due to its clinical significance and ability to cause disease, it has been the subject of many investigations. Several in-depth reviews have been recently published regarding the mechanism of action in renal cells (positive for cell surface globotriaosylceramide [$Gb3^+$]) (263), the intestinal epithelium ($Gb3^-$) (264), and the vascular endothelium ($Gb3^+$) (265) and its contribution to the pathophysiology of HUS (266).

Shiga toxins can be classed into two types, Stx1 and Stx2, with Stx1 having 3 subtypes (a, c, and d), and Stx2 having 7 (a to g) (267). STEC can carry a single variant, either stx_1 or stx_2, both stx_1 and stx_2, or a combination of stx_2 subtypes (e.g., stx_{2a} and stx_{2c}). Both stx_1- and stx_2-containing STEC can lead to HUS; however, stx_2 is more often associated with severe disease (252). It should be noted that not all subtypes have been isolated from human disease.

Both Stx1 and Stx2 are encoded on prophages that are integrated into the chromosome. Shiga toxin-carrying phages can become lytic during bacterial stress, and it is believed that Stx1/Stx2 is released from lysed bacterial cells during the lytic cycle of the phage (268). Clinically, the use of antibiotics to treat STEC infections has become contentious due to the stimulation of the lytic cycle and concomitant toxin release through the bacterial SOS response. Studies have shown that fluoroquinolones increased Stx2 production in STEC O157:H7 (269) and that subinhibitory concentrations of fluoroquinolones and trimethoprim induced the lytic cycle, while other antibiotics such as azithromycin did not (270).

Loss of the *stx*-containing phage has been reported in humans. Initially *stx*-positive STEC O26:H11/NM and SFO157:NM strains were isolated from patients; however, the isolates were *stx* negative

when isolated several days later from the same patient (271, 272). Since *stx*-negative STEC fits the definition of aEPEC, Bielaszewska and colleagues looked at isolates that were recovered from bloody diarrhea and suggested that they are not aEPEC, and they coined the term EHEC that lost the Shiga toxin (EHEC-LST) (273). Thus, the *stx* status of STEC can fluctuate (274). While initial work was done mostly on STEC O26:H11 and O157:H7/NM, additional serotypes, such as O103:H2/NM and O145:H28/NM, could also be isolated as EHEC-LST (188).

The mechanism of Stx delivery and trafficking through endothelial cells, such as renal cells, has been thoroughly described by Lee et al. (275). Briefly, Stx binds to Gb3 on the surface of endothelial cells (276, 277) and is internalized and trafficked through the retrograde pathway from the Golgi apparatus and endoplasmic reticulum (ER) and eventually to the host cell cytoplasm. The A subunit is an RNA-glycosidase that removes an adenine from 28S rRNA, thereby inhibiting protein synthesis and causing cell death. The mechanisms of how Shiga toxins causes cell death are complex, and involve several stress pathways that have been recently reviewed (278). Mechanisms of Stx transport from the intestinal lumen across the epithelium are unknown. It is hypothesized that STEC-induced inflammation may provide the toxin an opportunity to breach the epithelial barrier, and there are other possible mechanisms (discussed in reference 264). A recent study proposed that STEC is able to cross the intestinal epithelium through microfold cells (M cells) and survive in macrophages, and this may be a way for the Shiga toxin to be released into the bloodstream, where it can target other organs (279).

(ii) Cytolethal distending toxin. The cluster encoding the cytolethal distending toxin (*cdtABC*) can be found in many *E. coli* strains. It is found frequently in SFO157:NM isolates but not as often in STEC O157:H7 (280), where it has been associated with certain STEC O157:H7 phage types (281). Sequence differences between the four EPEC *cdt* variants relative to the one found in SFO157:NM have led it to be designated *cdt-V* (282). Once delivered into the cell, the enzymatically active CdtB is thought to trigger cell arrest by damaging host DNA (283). Bielaszewska et al. (282) confirmed that the Cdt-V variant from SFO157:NM was able to induce cell arrest in endothelial cells and may contribute to HUS (284). Cdt-V can be found in LEE-negative STEC serotypes O91:H21 and O113:H21.

(iii) EHEC hemolysin. The EHEC hemolysin (EHEC-*hlyA* or *ehx*) is a pore-forming toxin that lyses sheep erythrocytes (285, 286). Although the role of Ehx in virulence has been unclear, several investigations have led to some insight on its contribution to disease. These toxins have been shown to be associated as cargo with outer membrane vesicles (OMVs), prolonging its activity (287). Additionally, Ehx was found to be cytotoxic to endothelial cells and may contribute to the development of HUS (288). More recently, Ehx was shown to be inactivated by another STEC virulence factor, EspP (289). In STEC O113:H21, *ehx* is found on the large pO113 plasmid (247).

(iv) Autotransporters. The best studied autotransporter in STEC is the serine protease EspP. Of the four subtypes of EspP, only EspPα and EspPγ are secreted and active (290). EspPα is more common in STEC serotypes correlated with severe disease, such as STEC O157:H7, O26:H11/NM, O111:H8/NM, and O145:H25/H28/NM (290). EspP is a multifunctional protease that cleaves human coagulation factor V, pepsin A (291), complement (292), and EHEC hemolysin, inactivating its hemolytic activity (289).

(v) LEE-positive STEC. Undoubtedly, LEE-positive STEC has been the best characterized subset of STEC, and these strains form A/E lesions similar to those formed by EPEC (Fig. 6). Studies on pathogenesis have been focused on STEC O157:H7, with extrapolation of some LEE and Nle function derived from the EPEC literature. One striking difference between LEE-positive STEC and EPEC is the effector repertoire found in LEE-positive STEC; genome searches of the STEC O157:H7 genome identified 62 possible effector genes, with 13 thought to be pseudogenes (293), compared to the ca. 30 effectors from EPEC (94). While further studies demonstrated that only 33 of the STEC O157:H7 effectors were likely functional, many serotypes, such as O26, O103, and O111, have more effectors than O157:H7 (35). Generally, most effectors are conserved among all LEE-positive STEC strains, with only minor differences in repertoire.

The regulation of LEE expression is complex and is actively being studied in STEC O157:H7 (reviewed in reference 90). Of particular interest are the stimuli that LEE-positive STEC encounters during the course of infection to strategically control LEE genes. It has been shown that LEE-positive STEC is able to gauge its environment in different hosts through the detection of hormones and signaling molecules from both the host and the microbial flora and to regulate the LEE accordingly (reviewed in references 294 and 295). LEE-positive STEC has also been demonstrated to sense short-chain fatty acids, ethanolamine, and fucose to regulate LEE expression (296–298), further adding to the complexity of LEE regulation during colonization and pathogenesis.

(a) Attachment. The numerous adhesins encoded in the LEE-positive STEC genome likely contribute to colonization of the intestinal epithelium and other surfaces such as foods. Recent progress has been made in defining the contributions of these various structures and their potential in colonization. Fimbriae such as the *E. coli* YcbQ laminin-binding fimbria (ELF) and long polar fimbria (Lpf) have been shown to attach to the extracellular matrix (ECM) protein laminin (299, 300). Two *lpf* operons have been found in STEC O157:H7: *lpf1*, found on OI-141 ($lpf_{O157/OI-141}$), and *lpf2*, found on OI-154 ($lpf_{O157/OI-154}$) (301, 302). Interestingly, *lpf* mutants showed a change in tissue tropism and were able to colonize the small intestine in a human *in vitro* organ culture (IVOC) model (303). More broadly, *lpf1* or *lpf2* can be detected across all diarrheagenic *E. coli* pathotypes (304) as well as AIEC (305). Due to a number of *lpf* variants, a new classification scheme has been suggested by Torres et al. (306). Recently, a minor fimbrial protein, YadK, was shown to be involved in adherence to epithelial cells following exposure to acid (307).

The hemorrhagic coli pilus (HCP) is a type IV pilus found in STEC O157:H7 that forms long bundled fibers that are able to attach to extracellular matrix proteins on epithelial cells (308, 309). This pilus was also found to be multifunctional and contribute to phenotypes such as biofilm formation, twitching motility, and *in vitro* cell invasion (309), and it may trigger host immune responses (310). In SFO157:NM, the *sfp* operon is found on the pSFO157 virulence plasmid and was found to be involved in autoagglutination (311). Expression of the *sfp* under laboratory conditions required anaerobic conditions (312). Although *sfp* is gen-

erally thought to be restricted to SFO157:NM isolates, it has been found in STEC O165:H25/NM (313).

Other proteins that are thought to be involved in LEE-positive STEC adherence are Efa-1/LifA and its homolog, ToxB (314, 315). Unlike the EPEC *efa-1/lifA* products, STEC O157:H7 Efa-1 and ToxB do not have lymphostatin activity (316). Finally, the IrgA homolog adhesin (Iha), found in an OI with tellurite resistance, has adhesive properties and can also be found in LEE-negative STEC (317).

The autotransporter calcium-binding antigen 43 homolog (Cah) can promote cell-to-cell aggregation and biofilm formation (318). Similarly, the autotransporters EhaA and EhaB were also found to be involved in biofilm formation (319, 320). Contributions of Cah, EhaA, and EhaB in intestinal colonization remain unknown.

Intimate attachment is afforded by the interaction of intimin and Tir, similar to the case for EPEC as described above, except for some fundamental differences involving a separate effector that binds Tir and recruits host cell components that mediate actin assembly (reviewed in reference 321).

(b) StcE. StcE is a large metalloprotease that is secreted by the type II secretion system (encoded by *etp*), also found on the pO157 virulence plasmid (322). The first target identified for StcE was the C1 esterase inhibitor; cleavage of C1 esterase inhibitor is thought to prevent the activation of the classic complement pathway (323). Other targets for StcE include glycoprotein 340, mucin 7, and CD43 and CD45 on neutrophils (324, 325). Interestingly, the mucinase activity of StcE is hypothesized to protect STEC O157:H7 in the oral cavity by reducing the viscosity of saliva and preventing mucin-bacterial aggregates (324). Additionally, these authors propose that StcE activity aids in intimate attachment of LEE-positive STEC to intestinal epithelial cells by reducing the mucus barrier on the host cells.

(c) HPI. Originally identified in *Yersinia* spp., a high-pathogenicity island (HPI) was also discovered in the chromosome of STEC O26:H11/NM (326) and encodes the siderophore yersiniabactin. HPIs can be found infrequently in other pathotypes and commonly in EAEC (327).

(vi) LEE-negative STEC. It is becoming clear that not all STEC strains that cause HC and HUS are LEE positive, and thus their molecular mechanisms of disease are likely fundamentally different from those of their LEE-positive counterparts. Various LEE-negative STEC strains have been isolated from HUS patients, such as serotypes O91:H21 (328), O104:H4 (44), and O113:H21 (329). Analysis of the German STEC O104:H4 outbreak strain has shown that it is more closely related to EAEC (330), and the pathogenic mechanisms are likely similar to those of EAEC. Indeed, there have been reports of *stx*-expressing EAEC, and this is further discussed below in the section on enteroaggregative *E. coli*.

(a) Attachment. The mechanisms of epithelial cell attachment of LEE-negative STEC have not been studied as extensively as those of LEE-positive STEC. Nevertheless, several unique adhesins have been identified. Some LEE-negative STEC strains carry a single *lpf2* homolog, which is commonly referred to as lpf_{O113} due to its original discovery in STEC O113:H21 (331). STEC autoagglutinating adhesin (encoded by *saa*) was identified in an STEC O113:H21 isolate (332) and was subsequently determined to be present in various other LEE-negative STEC strains that have been isolated from patients with diarrhea or HUS (332, 333). The autotransporter protein Sab (an STEC autotransporter contributing to biofilm formation) was shown to contribute to adherence to epithelial cells and biofilm formation when expressed in laboratory *E. coli* (334). Like Saa, Sab was also found in other LEE-negative STEC strains and was also suggested to contribute to attachment and colonization of LEE-negative STEC strains (334). Finally, *E. coli* immunoglobulin-binding (Eib) protein G was identified near phage genes in the chromosomes of certain STEC O91 serotypes and contributes to a chain-like adhesion pattern (CLAP), which can vary in length, depending on the EibG subtype (335, 336).

(b) Invasion. Several other LEE-negative STEC serotypes, such as O113:H21, are able to be internalized and exist within a vacuole of epithelial cells *in vitro* (337). The flagellar antigen H21 contributes to invasion, and the invasive process may involve lipid rafts (338, 339).

(c) Activatable Stx2d. Some LEE-negative isolates, such as STEC O91:H21, carry a variant of Stx2 called $Stx2d_{activatable}$, which becomes more cytotoxic in the presence of mucus (340). In a potency study, $Stx2d_{activatable}$ was among the most cytotoxic Stx2 variants studied (341). A survey of different STEC serotypes found that $Stx2d_{activatable}$ was carried only by LEE-negative STEC and was associated with HC and HUS (342).

(d) Subtilase cytotoxin. The genes encoding the subtilase cytotoxin (*subAB*) were originally identified on a virulence plasmid in STEC O113:H21 (343); however, a variant can also be found chromosomally on a PAI in other STEC strains (344, 345). In Vero cell assays, recombinant SubAB was found to be more toxic than Stx1 or Stx2 (343). Pure SubAB given to mice is lethal and causes hemorrhaging in the small intestine and a disease similar to HUS, including renal damage, thrombotic microangiopathy, intestinal hemorrhages, hemolytic anemia, and thrombocytopenia (346, 347). Damage was also seen in different organs, such as the liver, spleen, and brain (347), and has been reported to cause leukocytosis (348).

The mechanism of SubAB toxicity and trafficking has been recently reviewed (349). Briefly, SubAB is internalized and trafficked to the endoplasmic reticulum. The catalytic A subunit is a serine protease that cleaves BiP/GRP78, a regulator of the stress response in the ER, and ultimately leads to cell death. SubAB may also be involved in altering host immune responses, as it was shown to prevent antibody secretion from B cells (350) and to suppress NO in macrophages, leading to the hypothesis that it may promote the survival of STEC in macrophages (351).

Clinical Considerations

Symptoms and onset of disease. Infections with STEC can range from mild watery diarrhea to bloody diarrhea (hemorrhagic colitis) and present a risk for the development of HUS. Both O157 and non-O157 STEC can have similar clinical presentations, but disease severity can differ between different serotypes, and hence a seropathotype classification was proposed (248). Typically, the incubation period before the onset of symptoms for STEC O157:H7 is about 3 days (352), which is similar to what was reported for an outbreak of STEC O111:NM (236). The first symptom is usually diarrhea, which can be accompanied by fever, abdominal cramping, or vomiting (reviewed in reference 353). Patients may experience hemorrhagic colitis in the following days after the initial onset of diarrhea. It is generally thought that STEC O157:H7 results in higher rates of bloody diarrhea than non-O157 STEC. A study in Montana found that bloody diarrhea occurred in 81% of STEC O157:H7 cases, compared to 58% of non-O157

STEC cases (354). A review by Johnson et al. also discussed the diversity of clinical data regarding rates of bloody diarrhea related to non-O157 illness (185). It has been recommended that patients with bloody diarrhea be hospitalized to help manage the diarrhea and prevent further communicable spread (353). Diarrhea caused by STEC O157:H7 generally resolves in about a week following initial onset, while non-O157 STEC diarrhea may persist longer (355). During the STEC O104:H4 German outbreak, the median shedding time was about 2 weeks in adults and more than a month in children under the age of 15 (356).

Despite the resolution of diarrhea, STEC illnesses are of particular concern due to the association of STEC infections with HUS (357). While STEC O157:H7 is a major cause of HUS worldwide, SFO157:NM and non-O157 serotypes such as O26, O103, O104:H4, O111, O121, and O145 have also lead to cases of HUS (44, 185, 186, 240). HUS involves thrombocytopenia, hemolytic anemia, and acute renal failure and can develop between days 5 and 13 after the initial onset of diarrhea (reviewed in reference 353). A 20-year epidemiological survey of 350 outbreaks in the United States determined that STEC O157:H7-associated HUS occurred in about 4% of cases and had a mortality rate of 0.5% (256). These numbers are much lower than the estimated HUS rate of 14% in children under the age of 10 (358), with a mortality rate of about 1 to 4% (359). Perhaps not surprisingly, the diversity of non-O157 STEC leads to variable HUS rates, albeit lower than those for STEC O157:H7 (185). In the unprecedented O104:H4 outbreak, HUS developed in about 20% of cases (44), while an SFO157:NM outbreak had an 11% mortality rate in children (240). Further long-term sequelae (in 20 to 40% of patients) from HUS include cardiac complications, gastrointestinal complications, neurological disorders, hypertension, chronic renal disease, cognition and behavior changes, and diabetes mellitus and have been recently reviewed elsewhere (359). It has been recommended that follow-up assessments for late-onset sequelae be done for at least 5 years (360).

Detection. Culture techniques have been previously described (8, 361), and the CDC has outlined methods of STEC isolation and identification along with recommendations in recent publications (362, 363), so these will only be briefly discussed here. Since most O157:H7 isolates are unable to ferment sorbitol within a 24-h period, they are easily distinguishable as clear colonies on sorbitol-MacConkey (SMAC) plates (364), although other chromogenic selective media have been shown to have fewer false-positives and may reduce operating laboratory costs (365). These identified colonies can then be checked for the O157 and H7 antigens using latex agglutination assays (366), which are available as commercial kits. Although uncommon in North America, SFO157:NM may not readily be identified from other sorbitol-fermenting *E. coli* strains and could be easily missed on SMAC plates. Additionally, the use of cefixime-tellurite (CT)–SMAC plates may also miss SFO157:NM isolates due to their sensitivity to tellurite (367) or may miss STEC O157:H7 in situations where OI-43 or OI-48 is lost due to deletions (368). For a strain to be classified as STEC, the Stx1 or Stx2 toxin must be detected, and this can be done through commercially available enzyme immunoassay (EIA) kits (362, 363). In serotypes O26, SFO157:NM, O103, and O145, it has been reported that *stx* can be lost from *in vivo* samples, and this may impact diagnostics (272, 274, 369). Because of the possibility of an *stx*-negative phenotype, a secondary target, such as intimin or, to differentiate SFO157:NM from O157:H7, *sfp* genes may be useful (188).

While STEC O157:H7 can usually be identified phenotypically on media, non-O157 STEC strains are not readily distinguishable from other *E. coli* strains because they are often able to ferment sorbitol, thus appearing as pink colonies on SMAC plates (363). Commercial enzymatic assays have been developed to increase the sensitivity of detecting both O157:H7 and non-O157 STEC compared to that with SMAC (370, 371). Although non-O157 STEC will be Stx1/Stx2 positive with EIA kits, further isolation is not routinely done in clinical laboratories (363).

Many methods have been developed for rapid identification of STEC from food sources and stool samples. Depending on the technique, these methods are able to indicate the serogroup and possible virulence factors held by particular isolates. With the extensive sequencing of the O-antigen-coding locus of various *E. coli* strains, serogroup-specific oligonucleotide pairs can be used in PCR assays (reviewed in reference 10). Serotyping methods have been developed to detect STEC using microarrays, matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS), and microbeads (372–374). Quantitative PCR (qPCR) panels have also been developed to look at multiple genes, such as *rfbE* (O157 antigen), *stx*, *eae*, *ehx*, *fliC*, and O-antigen genes to profile for certain STEC isolates (375). These methods are generally not approved for diagnosis from human samples but may be useful for epidemiological and outbreak studies by public health laboratories.

Recently, the CDC recommended Shiga toxin testing for the detection of non-O157 STEC and cultures for STEC O157:H7 of all stools submitted from patients with community-acquired diarrhea (362, 363). These recommendations, however, have not been adopted by all (376).

Treatment. The course of STEC infection usually is self-limiting and resolves after a week (355). Currently, there is no way to prevent the development of HUS following an STEC infection, and management of HUS has been discussed elsewhere (reviewed in reference 377). A recent publication by Wong et al. has identified risk factors that are associated with the development of HUS in children, including vomiting, a high leukocyte count, and use of antibiotics (358). Recent work has shown improved renal protection in children under the age of 18 against oligoanuria HUS when given intravenous fluids (378). Suggestions for clinical management of STEC infections were made by Holtz et al. (379), which include the use of intravenous fluids, renal function and platelet monitoring, and discouragement of the use of antimotility drugs, pain relief drugs, and antibiotics.

Alternative and experimental treatments. With no clear treatment of HUS, scientists and clinicians have been developing alternate methods that may provide useful treatment in the future, which have been recently reviewed by Goldwater and Bettelheim (380).

Monoclonal antibodies (MAbs) have been shown to reduce the cytotoxicity of Stx1 and Stx2 *in vitro* (381), while Urtoxazumab, a humanized MAb against Stx2, was shown to be safe in a phase 1 study (382). Similarly, phase 1 clinical trials of chimeric monoclonal Stx1- and Stx2-neutralizing antibodies were also shown to be generally well tolerated in injected volunteers (383, 384). Alternatively, short peptides may block Stx2 transport through epithelial cells (385). These cell-permeative peptides were also protective in a baboon model following Stx2 injection, as renal injury was abated (386). Cytotoxicity of both Stx1 and Stx2 could be neutralized with globotriose conjugated to the polysaccharide chitosan,

TABLE 4 Serotypes of EIEC

O serogroup[a]	H antigen(s)
O28ac	NM
O29	NM
O112ac (D2, B15)	NM
O115	NM
O121	NM
O124 (D3)	NM, H7, H30, H32
O135 (F2a)	NM
O136	NM
O143 (B8)	NM
O144	NM, H25
O152 (D12)	NM
O159	H2
O164	NM
O167 (B3)	NM, H4, H5
O173	NM

[a] *Shigella* serotypes that share the same or a closely related O antigen are indicated in parentheses. B, *S. boydii*; D, *S. dysenteriae*; F, *S. flexneri*.

TABLE 5 Serotypes and subserotypes of *Shigella*

Shigella species (subgroup)	Serotypes and/or subserotypes
S. dysenteriae (A)	1–15
S. flexneri (B)	1a, 1b, 2a, 2b, 3a, 3b, 4a, 4b, 4c, 5a, 5b, 6, X, Y
S. boydii (C[a])	1–20
S. sonnei (D)	NA

[a] *S. boydii* type 13 was recently reclassified as *E. albertii* (416).
[b] NA, not applicable.

by binding the toxin (387). This globotriose conjugate was also able to protect mice against STEC O157:H7 challenge. Synsorb-PK, a receptor analog, was designed to eliminate Stx1/2 from the intestine and was tested in clinical trials. Rates of HUS did not differ from those for controls, and the authors suggest that treatment may have been given too late in the progression of disease (388).

Other strategies that are being evaluated in the laboratory involve probiotics, such as secreted molecules from *Lactobacillus acidophilus*, that were able to inhibit STEC O157:H7 from adhering to epithelial cells *in vitro* and reduce shedding in mice (389). *Bifidobacterium* spp. and *Lactobacillus* spp. also were shown to be capable of inhibiting STEC O157:H7 growth in the laboratory (390). Manganese ions were shown to prevent cell death by Stx1 by preventing trafficking through the Golgi apparatus and protected mice against fatal Stx1 injections (391). A more recent paper, however, has challenged the use of manganese as a potential therapeutic against Shiga toxin (392).

Antibiotic resistance. Although antibiotics are not recommended for the treatment of STEC, many isolates carry resistance to various antimicrobials. Resistance to sulfonamide and tetracycline is common among O157:H7 and non-O157 STEC from both human and bovine sources (393). Furthermore, there is significant resistance to ampicillin, streptomycin, and trimethoprim, which can be more frequent in non-O157 STEC isolates. Plasmids have been found to be a source of antimicrobial resistance genes in STEC isolates (35, 394). Recently, an extended-spectrum β-lactamase (ESBL) plasmid, rarely found in STEC O157:H7, was isolated from a 2-year-old child in Denmark (395). An ESBL plasmid was also present in the STEC O104:H4 German outbreak strain (396). Tellurite resistance is also common in STEC O157:H7 and certain serotypes such as O26:H11/NM, O111:H8/NM, and O145:NM but is rare in SFO157:NM (317, 367, 397).

Vaccines. Vaccines are being developed against STEC (reviewed in reference 380). Some of these strategies have focused on reducing carriage and shedding in reservoir hosts such as cattle (398). Many studies have focused on protein fusions of different virulence factors. Some recent examples include fusions of Stx1B and an inactivated Stx2A (SAmB), Stx2B-Tir-Stx1B-zot (zot; mucosal delivery protein), Stx2B-Stx1B-initimin (SSI), and EspA-initimin-Stx2 (EIS), all of which have shown either reduced STEC shedding or protected against STEC or Stx1/Stx2 challenge in mice (399–402). Different delivery mechanisms for potential vaccines have also been explored, such as the EspA-intimin-Tir fusion that is expressed in tobacco leaves and canola seeds, *Salmonella* expressing intimin or EIS, and Stx2B expressed in the *Mycobacterium bovis* BCG vaccine strain (401, 403–405). Additionally, nontoxic Stx2A DNA vaccines have also been shown to provide some protection in mice (406).

ENTEROINVASIVE *E. COLI/SHIGELLA*

Enteroinvasive *E. coli* (EIEC) and *Shigella* spp. are facultative intracellular pathogens and the etiological agents of bacillary dysentery or shigellosis. *Shigella*, originally known as *Bacillus dysenteriae*, was first described by Kiyoshi Shiga in 1897 during an epidemic in Japan, where it infected more than 91,000 people and caused a mortality rate of greater than 20% (407, 408). EIEC was discovered about 50 years later and shares biochemical, genetic, and pathogenetic properties with *Shigella* (8, 409). In general, *Shigella* is nonmotile, lysine decarboxylase negative, and unable to ferment lactose (except for *S. sonnei*, a slow lactose fermenter). These and other characteristics also apply for most EIEC, with some exceptions that are described in more detail in "Detection" below (410, 411). Due to their high similarity, EIEC and *Shigella* are discussed here in parallel.

Classification

Common serotypes. EIEC comprises 21 major serotypes, which are typically defined by their O-antigen pattern, with a few exceptions that also present H antigens (410) (Table 4). Some of the EIEC O antigens are identical or closely related to *Shigella* O antigens and thus complicate the discrimination between EIEC and *Shigella* in conventional serotyping (412, 413) (Table 4). Traditionally, *Shigella* is classified based on biochemical, serological, and clinical phenotypes, not on phylogenetic correlation (411, 414, 415). It includes 49 sero- and subserotypes that are further clustered into the four species *S. dysenteriae* (serogroup A, 15 serotypes), *S. flexneri* (serogroup B, 14 sero- and subserotypes), *S. boydii* (serogroup C, 19 serotypes), and *S. sonnei* (serogroup D, 1 serotype) (Table 5). Until recently, *S. boydii* was subdivided into 20 serotypes; however, phylogenetic analysis revealed that *S. boydii* 13 belongs to the *E. albertii* lineage, which is distinct from typical *Shigella* (416).

Serotype classification provides the basis for the current *Shigella* and EIEC nomenclature. However, recent advances in phylogenetic analysis challenge this traditional classification scheme, which will be further discussed in the subsequent paragraph.

***Shigella*/EIEC: a distinct *E. coli* pathotype.** The genus name *Shigella* is still used, mainly for historical reasons and its associa-

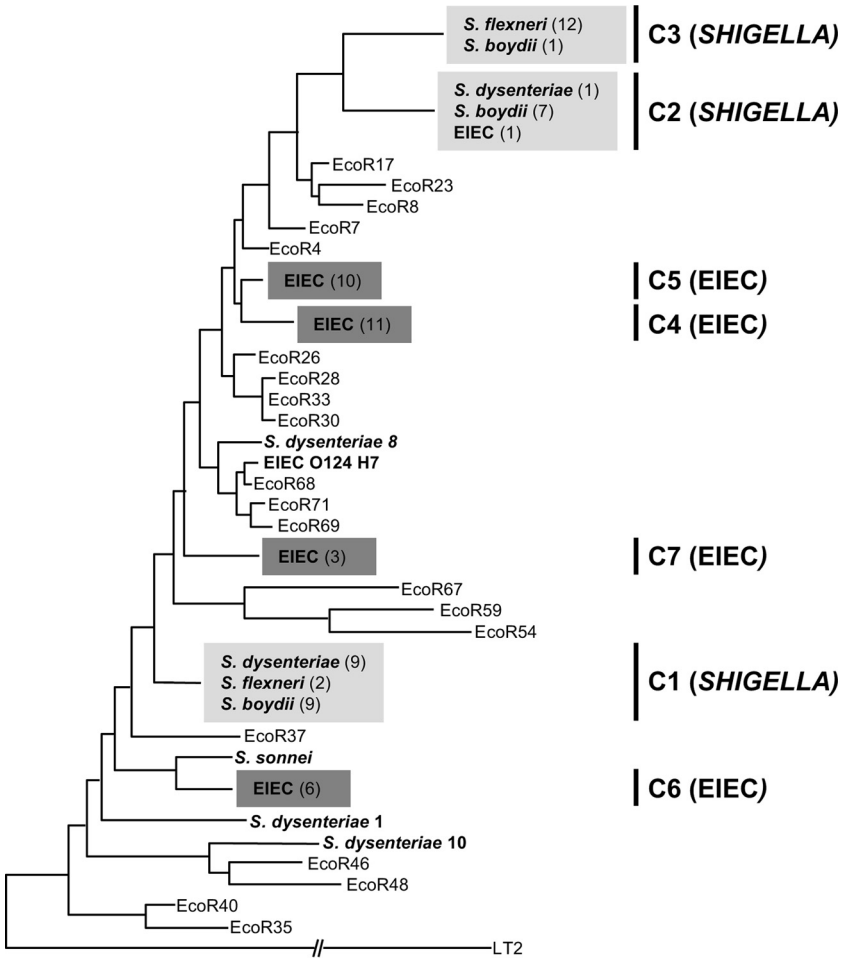

FIG 8 Phylogenetic tree of *Shigella*, EIEC, and nonpathogenic *E. coli* strains, showing the evolutionary relationship between 32 EIEC strains, 46 *Shigella* strains, and 20 *E. coli* reference strains of the ECOR group (EcoR strains) based on comparison of selected housekeeping gene sequences. *Shigella* groups in 3 phylogenetic clusters (C1 to C3) and EIEC in 4 phylogenetic clusters (C4 to C7), with broad distribution of traditionally surface antigen profile-classified strains. EIEC and *Shigella* strains outside clusters C1 to C7 are depicted in bold. Both EIEC and *Shigella* arose several times from multiple ancestral origins. The EIEC strain in C2 is assumed to be misclassified. The number of strains in a cluster is shown in parentheses. The *Salmonella* LT2 strain serves as outgroup. (Adapted from reference 409 with permission.)

tion with the disease shigellosis. However, diverse phylogenetic studies demonstrated that *Shigella* clearly belongs to the species *E. coli* (417–420). As exemplified by an analysis of 36 housekeeping genes from *E. coli* K-12, STEC O157:H7, and *S. flexneri* 2a, *Shigella* and the commensal K-12 strain showed an average sequence divergence of only 1.12% (417). In the same study, *Shigella* proved to be even more similar to *E. coli* K-12 than STEC O157:H7. Multilocus and whole-genome analyses revealed that EIEC and *Shigella* form a single pathotype within *E. coli* and emerged in several independent events from multiple ancestral origins (20, 409, 419, 421, 422). In a phylogenetic comparison study of 32 EIEC strains, 46 *Shigella* strains, and 20 *E. coli* reference strains of the *E. coli* Collection of Reference (ECOR) group, *Shigella* and EIEC strains, with few exceptions, formed three and four distinct clusters, respectively (409) (Fig. 8). Three out of four EIEC clusters contained strains with different O antigens, and strains sharing the same O antigen have been observed in more than one cluster. For *Shigella*, all clusters comprised strains of various traditionally defined serogroups. Thus, genetic relationships between strains are not comprehensively reflected by the commonly used traditional surface antigen profile-based classification scheme. EIEC showed less divergence from commensal *E. coli* than *Shigella*, which implies that EIEC arose later than *Shigella* and represents either a precursor of fully evolved *Shigella* or a form distinct from *Shigella*. This is in line with the reduced divergence observed within phylogenetic EIEC clusters compared to *Shigella* clusters (409).

So far, no common nomenclature exists for *Shigella* and EIEC that also incorporates the phylogenetic relationship between different lineages. Therefore, the traditional serotype-based nomenclature will be used for the rest of this section.

Pathoadaptation. Both the acquisition and loss of genes were necessary for *Shigella* and EIEC to evolve from being commensal *E. coli* with an extracellular lifestyle to being an intracellular pathogen that is fully adapted to the diverse environmental challenges within its host. These events have been well researched for *Shigella* and therefore are addressed here for *Shigella* in more detail. The transition from commensal *E. coli* to pathogen required the acquisition of novel virulence-promoting factors and the depletion or suppression of unnecessary and virulence-opposing genetic elements (Table 6). The extensive virulence toolbox of EIEC

TABLE 6 *Shigella* model of elements acquired or disposed of during pathoadaptation[a]

Adaptation	Category and element(s)	Role in pathogenesis[b]
Acquisition	Invasion plasmid elements	
	T3SS	Translocation of T3S effectors into host
	T3SS substrates/translocators	Subversion of host cell processes for bacterial invasion, intracellular survival, and intra- and intercellular motility
	Transcriptional regulators	Coordination of T3SS-associated gene transcription, stabilization of T3S effectors in bacterial cytosol
	Chaperones	Watery diarrhea, inflammation
	Enterotoxin ShET2	
	Chromosomal *Shigella* PAIs	
	SHI-1(including enterotoxin ShET1 and SPATEs Pic and SigA)	Watery diarrhea, mucus permeabilization, serum resistance and hemagglutination
	SHI-2/3	Iron acquisition, regulation of inflammation
	SHI-O	Host immune evasion
	SRL	Iron acquisition, multidrug resistance
	Toxin	
	Shiga toxin (primarily in *S. dysenteriae* 1)	Inhibition of host protein synthesis, induction of apoptosis
Loss or inactivation	Antivirulence genes	
	ompT	Inhibition of intra- and intercellular motility
	cadA (for EIEC often *cadC*)	Attenuation of PMN transepithelial migration, enterotoxin activity, and phagosomal escape
	nadA, *nadB*	Inhibition of PMN transepithelial migration, cell-to-cell spread, and T3SS
	speG	Decrease of oxidative stress tolerance
	argT	Inhibition of cell invasion
	Surface structures	
	Flagella, fimbriae	Activators of host immune system; flagella negligible due to acquired T3S effector-mediated, actin-based motility
	Diverse catabolic pathways	Redundant due to pathogenic lifestyle

[a] PAI, pathogenicity island; PMN, polymorphonuclear leukocyte; SPATEs, serine protease autotransporters from *Enterobacteriaceae*.
[b] See references 38 and 424.

and *Shigella* derived mainly from the acquisition of the invasion plasmid pINV and multiple other virulence-associated, mobile genetic elements (423, 424). Among these, the chromosomally located *Shigella* pathogenicity islands (SHIs) SHI-1 and SHI-2 (3), SHI-O, and SRL (*Shigella* resistance locus) contribute a plethora of additional virulence factors and resistance to several antibiotics, which played a critical role during pathogen evolution (424). The highly dynamic *Shigella* genome harbors 300 to 650 additional insertion sequence elements compared to *E. coli* K-12 and thus affords the pathogen its pathogenic flexibility (425). In addition, the *Shigella* genome comprises numerous deletions (black holes) and more than 200 pseudogenes (425–427). Many metabolic pathways became dispensable due to the intracellular lifestyle of *Shigella* (424), especially the inactivation and depletion of genes that encode bacterial antivirulence factors that otherwise would oppress *Shigella*/EIEC pathogenicity, and are regarded as crucial elements of pathoadaptation; these were attained by convergent evolution and were comprehensively reviewed recently (38). In contrast to the acquisition of novel virulence factors, the identification of antivirulence genes that have been silenced by deletion, insertion, or point mutations is more challenging. Nevertheless, several inactivated antivirulence genes have been identified in EIEC and *Shigella*. Among these, *nadA* and *nadB* encode enzymes of the NAD synthesis pathway that catalyze the synthesis of quinolinic acid, an inhibitor of virulence. In contrast, *ompT* encodes a protease that directly triggers proteolytic degradation of VirG, a crucial effector for intracellular motility. Moreover, silencing of the *speG* gene results in the beneficial accumulation of spermidine, which increases resistance to oxidative stress encountered during infection. ArgT was observed to attenuate *Shigella* virulence and consequently was found to be downregulated at 37°C, the temperature of the host environment. Finally, lysine decarboxylase (LDC) is an important virulence-attenuating factor that is absent in both EIEC and *Shigella*. For *Shigella*, it has been shown that a product of lysine decarboxylation, cadaverine, attenuates the activity of bacterial enterotoxins, inhibits the migration of polymorphonuclear leukocytes across the epithelial barrier, and blocks phagosomal escape (426, 428, 429). All of these are important elements of EIEC and *Shigella* virulence. LDC is encoded by the gene *cadA*, which is part of the *cadBA* operon and under positive control of the regulator CadC. EIEC and *Shigella* reveal different strategies to block LDC synthesis. In most *Shigella* strains, the *cadA* gene is either deleted or inactivated, whereas most EIEC strains still possess *cadA* but have an inactivated *cadC* (426, 430).

Epidemiology

Transmission and reservoirs. Conventional host-to-host transmission of EIEC and *Shigella* is mediated via the fecal-oral route mainly through contaminated water and food or direct person-to-person spread (431, 432) (Fig. 5). In addition, flies and living amoebae have been reported to play a role in *Shigella* transmission (433, 434). Disease is caused in humans and higher nonhuman primates with an infectious dose of as little as 10 to 100 CFU of *Shigella*, as observed in volunteer studies (435). EIEC infection tends to be less effective, and higher infectious doses have been reported from volunteer studies (436).

Incidence and outbreaks. EIEC is underrepresented in epidemiological surveys due to its less severe clinical manifestations. In addition, EIEC strains with very close biochemical, genetic and pathogenic similarity to *Shigella* might be misclassified, whereas EIEC strains with commensal *E. coli* characteristics, such as lactose fermentation, might remain undetected in respective surveys. Hence, no specific geographical pattern can be associated with EIEC incidences, as the epidemiology of EIEC can be properly addressed only by studies that are more specifically designed for EIEC, which would include EIEC-specific genetic markers or laborious EIEC typing.

No EIEC episodes have been reported in recent health-related government surveillance programs led in the United States, Canada, Europe, or Australia (193, 195, 437, 438). However, rare cases do occur, including a 1985 food-borne EIEC outbreak which affected 370 people in Texas, United States (439). In the last decade only a few disease-associated EIEC episodes with more than 10 cases have been reported from studies in Central and South America, Africa, and Asia (65, 440–443).

In contrast, the epidemiology of *Shigella* is well documented. *Shigella* causes diarrhea and is associated with about 30 to 50% of bacillary dysentery cases worldwide (444). A study in 1999 estimated 164.7 million *Shigella* episodes annually, with 99% affecting developing countries, including 1.1 million deaths per year. Approximately two-thirds of episodes and deaths involved children under 5 years old (445). More recent studies have reported a declining trend of the *Shigella* disease burden, a common trend for diarrheal diseases in general (5, 446, 447). This is more likely a consequence of the dramatic drop in case-fatality rates, which is also reflected by the last WHO figures in 2009 estimating 90 million *Shigella* episodes and 108,000 deaths per year worldwide (http://www.who.int/vaccine_research/diseases/diarrhoeal/en/index6.html), than of changes in overall morbidity. Improved primary health care, education, and hygienic standards in areas of endemicity and the absence of devastating *S. dysenteriae* 1 epidemics are plausible reasons for this recent development (448). Nevertheless, *Shigella* still remains a major public health concern, with a high mortality rate, evolving epidemiology, increasing multidrug resistance, and a high incidence of unreported cases. GEMS reported that *Shigella* was among the four major pathogens associated with moderate to severe pediatric diarrhea, being the fourth most common isolate among infants of 0 to 11 months, second among toddlers of 12 to 23 months, and first among children of 24 to 59 months (7).

All *Shigella* species are covered by most epidemiological studies. The Shiga toxin-producing *S. dysenteriae* 1 was responsible for the high mortality rate during devastating pandemics in Central America in the 1960s, South Asia in the 1970s, Central Africa in the 1980s, and East Africa in the 1990s (449–452). The last major *S. dysenteriae* 1 epidemic was recorded in Sierra Leone, West Africa, in 1999 (453). Shigellosis due to *S. dysenteriae* serotypes other than type 1 is uncommon. *S. boydii*-mediated disease also is rare and mainly restricted to the Indian subcontinent (447). In contrast, little geographical restriction applies for *S. flexneri*, which is the most frequently isolated *Shigella* sp. worldwide and is endemic in developing countries. A literature-based study in 2007 incorporating countries in Asia, Africa, and South America demonstrated that in most places, *S. flexneri* was responsible for about two-thirds of shigellosis cases, with *S. flexneri* 2a being the single most prevalent subserotype (454). More recent reports from India, Pakistan, Bangladesh, Argentina, and China confirm *S. flexneri* as the most prominent *Shigella* sp. being detected (455–458). However, a major shift from *S. flexneri* to *S. sonnei* is becoming more evident in transition countries and areas with improving socioeconomic conditions (454, 459, 460). In developed countries, *S. sonnei* is the most prevalent *Shigella* species and causes sporadic disease and outbreaks in epidemiological niches such as day care centers (461). In general, the average *Shigella* incidence rate in developed countries ranges from 1.6 to 4 cases per 100,000 population according to recent estimations by surveillance programs in the United States, Canada, Europe, and Australia (193, 195, 437, 438). *Shigella* infections that affect travelers and workers from industrialized countries who entered areas of endemicity account for 2 to 8% of traveler's disease (1 to 2% for EIEC) and usually involve *S. flexneri* and *S. sonnei* (462). Changing traveling habits in a rapidly globalizing world are an important aspect affecting the propagation of pathogenic agents. Holt et al. recently performed a phylogeographic analysis based on results from whole-genome sequencing of 132 globally distributed *S. sonnei* isolates that have been collected over the course of 60 years. They showed that most current *S. sonnei* infections are caused by only a small number of successful, often antibiotic-resistant strains that originally disseminated from Europe and spread globally (463). This rapid global dispersion of robust and infectious strains illustrates that *Shigella* is still a massive global health threat with rapidly evolving multidrug-resistant strains.

Pathogenesis

EIEC and *Shigella* are highly invasive pathogens that use the intracellular milieu of intestinal epithelial cells (IECs) in the large intestine as their replicative niche. These pathogens are readily adaptable to the various environmental challenges they face during the course of infection, including low gastric pH, temperature changes, oxygen availability, and oxidative stress, as well as osmolarity (464). Advances in *S. flexneri* research provide the basis for our current understanding of both EIEC and *Shigella* pathogenesis at the cellular and molecular levels, which has been comprehensively summarized in recent reviews (424, 465). Successful infection is dependent on essential virulence determinants that are encoded by both chromosomal and plasmid loci. Key plasmid-encoded virulence factors include components of the T3SS needle complex (Mxi-Spa proteins), chaperones (IpgA, IpgC, IpgE, and Spa15), transcriptional regulators (VirF, VirB, and MxiE), translocators (IpaB, IpaC, and IpaD), and approximately 25 effector proteins. An additional 5 to 7 T3SS substrates of the IpaH family and further T3SS-independent virulence factors are chromosome encoded (424). *Shigella* infection is a multistep process involving penetration of the epithelial barrier, induction of macrophage cell death, IEC invasion, suppression of the immune response, intra- and intercellular movement, and modulation of epithelial integrity (Fig. 6).

Penetration of epithelial barrier and macrophage cell death. In order to gain access to the basolateral surface of IECs, bacteria first penetrate the epithelial barrier through M cells by transcytosis. In the underlying submucosa, *Shigella* is phagocytosed by resident macrophages and evades its own degradation by effector-dependent phagosomal escape and induction of caspase I-dependent pyroptosis-like macrophage cell death (466–468).

Invasion. *Shigella*'s subsequent basolateral invasion of IECs, its intracellular survival and replication, and cell-to-cell spread are governed by the ability of its effector repertoire to subvert host cell signaling pathways (424). Bacterial adhesion to the host cell is

mediated by IpaB and an IpaBCD complex, which bind to the host hyaluronan receptor CD44 and $\alpha_5\beta_1$ integrin, respectively (469, 470). IpaC, IpgB1, IpgD, IpaA, and VirA have been implicated in extensive host cytoskeleton reorganization and membrane ruffling to promote pathogen uptake into the phagosome (471–476). Subsequently, *Shigella* escapes the phagosome by utilizing the effectors IpaB, IpaC, IpaD, and $IpaH_{7.8}$ (477–480).

Suppression of host immune response. Multiple effectors counteract the host immune defense (481). Host inflammatory responses, such as the mitogen-activated protein kinase (MAPK) and NF-κB pathways, as well as cytokine production, are targeted and dampened by multiple effectors (481), including OspG, OspF, OspB, OspZ, OspI, $IpaH_{4.5}$, and $IpaH_{9.8}$ (126, 482–488). Additionally, *Shigella* employs strategies to manipulate and escape the host immune response, such as suppression of antimicrobial peptide expression, induction of apoptosis in dentritic cells, and impairment of T-cell function (489–491). Moreover, the T3S effectors VirA and IcsB help *Shigella* to circumvent its autophagy-mediated degradation (492, 493).

Intra- and intercellular movement. T3S effectors are responsible for the intra- and intercellular movement of otherwise nonmotile *Shigella*. VirG induces the acquisition of N-WASP, which then leads to the formation of an ARP2/3 complex-mediated unipolar actin tail on the bacterial surface. This tail provides the propulsive force required for directed motility of the pathogen (494, 495). Furthermore, it has been suggested that VirA, with its capability to stimulate microtubule destabilization, is another essential effector that allows *Shigella* to efficiently spread through the dense intracellular cytoskeletal network (496). The acquired motility also helps the pathogen to disseminate into neighboring cells, which was recently found to occur primarily at tricellular tight junctions (497).

Epithelial integrity. Epithelial integrity plays a fundamental role during *Shigella* infection (498). Early in infection, *Shigella* promotes host cell survival and integrity. Inhibition of IEC turnover and detachment by IpaB and OspE, respectively, and IpgD- or VirA-mediated blockage of host cell death are known mechanisms employed by the pathogen to conserve its replicative niche and promote colonization (499–502). However, *Shigella* has also evolved strategies to breach the epithelium to facilitate access to the basolateral surface of IECs and ultimately disembarkation from its replicative niche. This includes processes such as cell surface manipulation by IpaB-mediated disruption of Golgi network-driven secretion, destabilization of epithelial tight junction sealing, and induction of IEC death (503–505). The massive inflammatory response associated with apoptotic macrophages and IEC invasion, including infiltrating polymorphonuclear leukocytes, further perforates the epithelial barrier and ultimately leads to tissue lesions, which are characteristic for the pathology of shigellosis (424, 506).

Toxins. Additional virulence factors are known to contribute to the clinical manifestations of EIEC/*Shigella* infections. The common observation of watery diarrhea has been attributed to *Shigella* enterotoxins 1 and 2 (ShET1 and ShET2) (507–509). The chromosome-encoded ShET1 is limited to *S. flexneri*, whereas the pINV-encoded and T3S ShET2 has been found in *Shigella* strains of different serotypes and EIEC (510–512). In addition to its contribution to secretory intestinal activity, ShET2 has also been implicated in *Shigella*-induced inflammation in IECs (510). Besides ShET1 and ShET2, two additional enterotoxigenic factors, namely, Pic, which is encoded by a chromosomally located gene that comprises the open reading frame of ShET1 on its opposite strand, and the pINV-encoded SepA, have been recently described to account for intestinal secretion in a mouse tissue model (513, 514). Both Pic and SepA belong to the family of serine protease autotransporters of *Enterobacteriaceae* (SPATEs). SPATEs are serine proteases that engage the autotransporter pathway and self-cleavage for their secretion. *Shigella* encodes two more members of the SPATE family, the cytotoxins Sat and SigA. SigA was found to contribute to intestinal fluid accumulation in the rabbit ileal loop model (515). SPATEs are not limited to EIEC and *Shigella* but are commonly found in a multiplicity of pathogens, including pathotypes described here, and are summarized in a recent review (516). Lastly, the often severe and lethal complications of *S. dysenteriae* 1 infections are associated mainly with Stx. Stx is mostly only present in *S. dysenteriae* 1 and is almost identical to Stx1 produced by other STEC strains. Besides its inhibitory impact on host protein synthesis by catalytic inactivation of eukaryotic ribosomes, Stx has been described to induce apoptosis in various cell types. As such, it was found to be responsible for the development of vascular lesions in the colon, kidneys, and central nervous system (517–519). In contrast to the case for STEC, the original Stx-encoding bacteriophage of *S. dysenteriae* 1 lost its functionality and thus transducibility (520). An *S. sonnei* isolate that carried a phage-encoded *S. dysenteriae* 1-like Stx was reported, revealing that other *Shigella* serotypes may have the potential to acquire Stx via horizontal gene transfer and thus increase their virulence (521).

EIEC versus *Shigella*. In general, EIEC and *Shigella* employ the same strategies to invade host cells. Nevertheless, EIEC exhibits reduced virulence compared to that of *Shigella*, including reduced expression of virulence genes, less efficient macrophage killing, reduced cell-to-cell spread, and decreased induction of a proinflammatory host response (522, 523), which correlates with the less severe disease induced by EIEC.

Clinical Considerations

Symptoms. The clinical presentation, progression, and complications of bacillary dysentery, or shigellosis, vary depending on the infectious agent, the immunological background of the host, and available medical infrastructure. The reader is referred to earlier literature for a more detailed overview of clinical symptoms (524, 525). Briefly, mild watery diarrhea, fatigue, malaise, fever, and anorexia develop in the early stages of disease. This is typically accompanied by abdominal cramps, tenesmus, scanty stools with blood and mucus, and dehydration. In most cases, shigellosis is self-limiting. However, severe and life-threatening complications can occur, especially in areas of the developing world where the disease is epidemic or endemic, where mainly young, often malnourished and strongly immunosuppressed children are infected and lack access to adequate treatment. Severe shigellosis complications are often associated with the Shiga toxin-producing serotype *S. dysenteriae* 1 and can range from local intestinal disorders to systemic manifestations. Shigellosis complications include toxic megacolon, intestinal perforation, peritonitis, hyponatremia, hypoglycemia, pneumonia, and HUS. HUS was estimated to occur in up to 13% of *S. dysenteriae 1*-infected, dysenteric patients, with a case-fatality rate of about 36%, in Africa and Asia (526). *S. dysenteriae* 1-caused HUS is very similar to HUS caused by STEC (see discussion in the section on Shiga toxin-producing *E. coli*

above). However, disseminated intravascular coagulation with consumption of coagulation factors can be present in *S. dysenteriae* 1-associated HUS cases but is rare with STEC (527). Further shigellosis complications include septicemia and neurological disorders, such as encephalopathy and seizures (528, 529). Incidences of postinfectious irritable bowel syndrome after *Shigella* infection have also been reported (530).

EIEC infection often leads only to self-limiting, mild watery diarrhea. However, in rare situations it can cause shigellosis-like symptoms (8). Complications have been reported, but are very uncommon (531).

Detection. A classical approach to identify EIEC and *Shigella* in a stool sample begins with the determination of invasiveness of the bacterial isolate. For this purpose, the guinea pig keratoconjunctivitis (Sereny) test, which requires prior isolation of the bacterial species, and the microscopic detection of numerous polymorphonuclear leukocytes in blood-containing stool samples are two possible phenotypic assays (525, 532, 533). In addition, a tissue culture plaque assay that exploits the ability of *Shigella* to form plaques in epithelial cell monolayers can be used to test invasiveness (534). However, the Sereny test is laborious and involves animal work, the plaque assay requires appropriate infrastructure, and both assays do not result in an unambiguous identification of either EIEC or *Shigella*. Therefore, alternative diagnostic tools are necessary and are often based on the biochemical distinction of EIEC and *Shigella* from other bacteria. For efficient diagnosis, the bacterial isolates need to be obtained from either fresh stool samples or stool specimens that were stored in adequate transport medium, such as buffered glycerol saline or Cary-Blair transport medium (524). Biochemical identification of *Shigella*, which can often be employed for EIEC, uses differential/selective media and focuses mostly on the following characteristics of almost all *Shigella* strains: inability to ferment lactose and utilize citrate; absence of motility, lysine decarboxylase, and urease activity; and acid but no gas and H_2S production upon sugar fermentation (447, 524, 535). If adequate equipment and scientific training are available, then PCR-based detection of pathotype-specific genetic markers, such as the invasion plasmid antigen H gene (*ipaH*) or the invasion-associated locus gene (*ial*), is a common diagnostic tool for both EIEC and *Shigella* detection (536). The latter is frequently performed as a multiplex PCR to simultaneously identify EIEC/*Shigella* and other pathogenic agents (537, 538). The recent development of a TaqMan Array Card platform, which is potentially able to multiplex up to 384 targets and was used to detect 19 pathogens, including 5 viruses, 9 bacteria (including EIEC/*Shigella*), 3 protozoa, and 2 helminths, represents a prospective tool for fast and comprehensive surveillance data processing on the next level of multiplexing technologies (539). Once the bacterial isolate is identified as EIEC or *Shigella*, the serogroup and serotype, respectively, can be confirmed by slide agglutination tests using commercially available antisera (447, 524, 535). Alternative approaches to antiserum-based serotyping have also been developed. Coimbra et al. designed a software-supported and restriction fragment length polymorphism-based technique which is able to distinctly identify most but not all *Shigella* serotypes (540, 541). In another study, molecular serotyping of *S. flexneri* was achieved by a multiplex PCR assay targeting several specific O-antigen synthesis and modification genes (542). The analysis of single nucleotide polymorphisms also has been employed to successfully determine *Shigella* serogroups (543). The increasing availability of genomic sequencing data facilitates the search for novel genetic markers that can be used for unambiguous *Shigella* and EIEC serotyping. In locations where the appropriate technological infrastructure is available, additional genome-based typing methods for precise assessment of clinical isolates and are on the rise thus provide a major contribution to identification, surveillance, and risk assessment of EIEC and *Shigella*. MLST constitutes a valuable tool to identify clinical isolates and characterize their degree of genomic relationship (13). By June 2013, the sequence types of 50 EIEC and more than 140 *Shigella* strains were listed and available in an open-access database (20; http://www.mlst.net). Comparative genomics represents an effective measure in the proper assessment of pathogenic isolates. For example, the high discriminatory power of a retrospective whole-genome analysis of an *S. sonnei* outbreak allowed the determination of various phylogenetic lineages and proved to be superior to conventional typing techniques in defining the outbreak (544). However, DNA sequence-based typing approaches are often difficult to implement in the field, where technological restrictions apply. The introduction of dipsticks has proven to be an applicable approach for rapid diagnosis of *S. flexneri* 2a and *S. dysenteriae* 1 in the field (545, 546). This format detects serotype-specific lipopolysaccharide (LPS) in less than 15 min by using serotype-specific monoclonal antibodies that are coupled to gold particles and displayed on a one-step immunochromatographic dipstick.

Due to their phenotypic, biochemical, and genetic relatedness it is difficult to distinguish between EIEC and *Shigella*. Generally, *Shigella* tests negative on motility, lactose, and mucate fermentation and L-serine, D-xylose, and/or sodium acetate utilization. Some EIEC strains test positive in one or more of these categories and can thus be distinguished from *Shigella* (417). However, these are not very reliable methods, since most EIEC strains would also show a negative *Shigella*-like phenotype. Recently, van den Beld and Reubsaet proposed a key for EIEC and *Shigella* identification combining IpaH gene PCR with several biochemical assays (547). In this detection strategy scheme, the authors suggested that the use of agglutination tests using EIEC- or *Shigella*-specific antisera is often unavoidable in order to clearly discriminate between EIEC and *Shigella*.

Some antisera cross-react with O antigens of both EIEC and *Shigella* (412, 413) (Table 5). In these cases the PCR-based detection of the lysine decarboxylase *cadA* gene, which is mostly absent in *Shigella* but often present in EIEC, may provide better distinction (413, 430). In general, genotypic analyses, such as MLST, provide higher resolution, which allows discrimination between *Shigella* and EIEC.

Treatment. The essential steps for effective treatment of shigellosis have been extensively reviewed and slightly adapted in the last decade (444, 524, 525, 548). Generally, mild to moderate shigellosis is considered to be self-limiting provided that proper rehydration is guaranteed. This essential first step in diarrhea therapy is manifested in a formula for effective oral rehydration therapy, which has been developed by the WHO (525). As a second step, antimicrobial treatment has proven to effectively shorten the duration of symptoms and to reduce the risk of serious complications and death (524, 549). In contrast to the case for STEC, early antimicrobial treatment was also reported to be efficient in reducing the risk of *S. dysenteriae* 1-associated HUS (550). In general, the severity of disease, age of the patient, and local antibiotic susceptibility pattern should be taken into account for an adequate

antibiotic-based strategy. The WHO guidelines for antibiotic treatment of shigellosis from 2005 were slightly revised recently (444, 525, 548). Recommended antibiotics for pediatric treatment include the macrolide azithromycin, the third-generation cephalosporin ceftriaxone, and the fluoroquinolone ciprofloxacin. Fluoroquinolones and azithromycin are recommended for adult treatment. The milder clinical manifestations of EIEC infection often do not require antibiotic treatment and are self-limiting (551). However, in the rare case of severe symptoms, an antibiotic strategy similar to that for shigellosis is recommended (548). In addition, zinc supplementation has been recommended due to its beneficial effect in reducing the severity and duration of diarrheal diseases in general (525, 552). Also, nutritional therapy with green plantains has been reported to be health supportive by increasing the weight gain of the diarrheal patient while decreasing the duration of the disease (553, 554).

Antibiotic resistance. Unrestricted use of antibiotics as a therapeutic agent has caused a dramatic increase in multidrug resistance among *Shigella*, an alarming fact with regard to the future treatment of dysentery (447, 555). High incidences of antibiotic resistance among *Shigella* were reported as early as the 1950s in a case of dysentery due to sulfonamide-resistant *S. sonnei* (556). Since then, *Shigella* has acquired resistance to all first-line antibiotics, including sulfonamides, ampicillin, tetracycline, chloramphenicol, sulfamethoxazole/trimethoprim, and nalidixic acid (557–560). Although less reported due to its lower incidence rate and morbidity, EIEC seems to follow a similar trend. A comprehensive analysis of both *Shigella* and EIEC strains that were collected from North and South America, Africa, and Asia between 1970 and 2000 showed tetracycline resistance within 70 to 86% of *Shigella* isolates and 48% of EIEC isolates (561). Also, resistance to all first-line antibiotics was reported for an EIEC isolate in Japan (562). Even more alarming is the increasing occurrence of fluoroquinolone resistance, especially among *S. flexneri* but also within other *Shigella* subgroups (563). Up to 6% of ciprofloxacin-resistant *Shigella* strains were isolated from diarrhea episodes in Asia between 2000 and 2004 (447). More recent studies revealed an occasional ciprofloxacin resistance rate of up to 10% in Asia (457, 564). In addition, one study in China reported a decrease in susceptibility to ciprofloxacin in approximately 80% of isolates (564). In contrast, resistance against the two other current therapeutics of choice, azithromycin and ceftriaxone, is still rare, but reports of sporadic incidences are on the rise (457, 565, 566). Antibiotic resistance in EIEC/*Shigella* is most commonly plasmid and/or chromosome borne (424, 567). The acquisition of mobile elements, such as the *S. flexneri* chromosomal pathogenicity island "*Shigella* resistance locus," which encodes resistance to streptomycin, ampicillin, chloramphenicol, and tetracycline, plays a crucial role in the fast evolution of drug-resistant strains (568). Alternatively, plant-based therapeutic approaches such as use of the *Aegle marmelos* fruit lectin or the essential oil from the Brazilian medicinal plant *Cymbopogon martinii* have shown promising antimicrobial activity *in vitro* (569, 570).

Despite some promising alternatives being in development, antibiotic treatment still remains the most effective treatment against EIEC and *Shigella*. Controlled and considerate use of antibiotics that also takes into account the regional antibiotic susceptibility patterns of EIEC and *Shigella* will slow down the emergence of multidrug-resistant strains. The improvement of health education, sanitary conditions, and access to clean drinking water, especially in impoverished areas of endemic diarrhea, is an important step toward reduction of *Shigella*- and EIEC-mediated disease.

Vaccines. An effective vaccine would constitute another preventive and sustainable approach to eliminate the disease burden of bacillary dysentery. However, no vaccine is currently available for either EIEC or *Shigella*. The development of an effective *Shigella* vaccine has therefore been the focus of many laboratories over the last decade. The progress made and the hurdles encountered in the development of a *Shigella* vaccine have been well documented in recent reviews (454, 571, 572). Briefly summarized, recent research pursues the design of a multivalent vaccine protecting against the most prevalent serotypes and subserotypes, including *S. dysenteriae* 1, *S. sonnei*, and all 14 types of *S. flexneri*. Multiple strategies were implemented to engineer both parenteral and mucosal candidate vaccines that have shown various levels of efficacy in clinical trials. The most promising candidates include live attenuated strains of *S. flexneri* 2a, *S. sonnei*, and *S. dysenteriae* 1, as well as inactivated whole-cell vaccines derived from inactivated *S. sonnei* and *S. flexneri* 2a strains. Subunit-based approaches involve covalent and noncovalent O-polysaccharide–protein conjugates targeting *S. flexneri*, *S. sonnei*, and *S. dysenteriae*, LPS-Ipa-protein complexes protecting against *S. flexneri* 2a and *S. sonnei*, and *S. flexneri* 2a-directed outer membrane vesicles. These candidates, together with major efforts to increase the immunogenicity of mucosal vaccines as well as the selection and design of potent adjuvants and antigen carriers, promise fast progress toward a long-awaited safe and powerful vaccine against *Shigella* (571, 573). Unfortunately, various factors have hindered a rapid solution thus far (454). The diversity of epidemiologically relevant *Shigella* serotypes, the fragile nature of its pediatric target group in developing countries, and the geographic divergence of shigellosis incidence, including different diets, sanitary standards, and target groups with various immunological backgrounds, are just some of the hurdles that complicate vaccine development process.

ENTEROAGGREGATIVE *E. COLI*

Enteroaggregative *E. coli* (EAEC or EAggEC) was identified by comparing adherence patterns of over 500 isolates from a case-control study in 1987 (574). Since its initial identification, EAEC has been identified in worldwide endemic and epidemic diarrheal diseases (575), and in several large-scale studies it has even been found to be the most common bacterial pathogen identified in diarrheal stool samples (576, 577). It causes persistent diarrhea in children in areas where EAEC is endemic (578) and persistent diarrhea in human immunodeficiency virus (HIV)-infected patients (579), and it is a causative agent for traveler's diarrhea (580).

Classification

After EAEC was first described, a method to identify the pathotype was developed using a probe that was later found to hybridize with an ATP-binding cassette transporter apparatus that translocates dispersin across the bacterial cell membrane (577, 581, 582). The majority of these probe-positive samples were also found to carry *aggR*, but not all diarrheagenic strains were positive for *aggR*, which led to a general classification of EAEC into typical (containing *aggR*) and atypical (lacking *aggR*) groups (583). Further classification of EAEC is possible based upon adherence patterns, as

some strains preferentially infect the small bowel, while others infect both the small bowel and colon (580).

In a study of 143 EAEC isolates gathered from outbreaks, sporadic cases of diarrhea, and hospital-based studies from 1985 to 2006 in the United Kingdom, 43 could not be serotyped (584). Of those that could, the most common serotypes isolated were O126:H27 (5 isolates), O44:H18 (6 isolates), O111ab:H21 (8 isolates), O73:H18 (2 isolates), O92:H33 (2 isolates) O126:H27 (2 isolates), and O136:H2 (2 isolates) (584). However, O serotyping of EAEC for the purposes of classification is problematic, as many strains autoagglutinate, there are a variety of serotypes, and even EAEC strains that share serotypes differentially adhere to HEp-2 cells, which is a common method used for detection and classification of different *E. coli* pathovars (584–586). Using MLST on 126 *E. coli* isolates from a Nigerian case-control study, researchers recently concluded that the sequence type complexes present in the Nigerian study were shared with well-studied EAEC strains from other parts of the world (16). Additionally, they found that the category of EAEC encompasses multiple pathogenic lineages, emphasizing the global heterogeneity of EAEC (16). Importantly, they concluded that no single strain can be considered representative of EAEC and suggested that to properly study EAEC, researchers instead must first identify specific virulence genes and mechanisms within these separate lineages.

Epidemiology

Incidence and outbreaks. As global surveillance for all strains of diarrheagenic *E. coli* was limited in the past, with much of the historical data regarding outbreaks of EAEC limited to North America, Europe, and South America, a comprehensive picture of EAEC incidence and outbreaks is still an ongoing research goal. However, EAEC was found to be the most common bacterial cause of diarrhea in the emergency departments and outpatient clinics of two large academic hospitals in Maryland and Connecticut in a large-scale study (577). EAEC has caused many significant outbreaks, including a 1997 school lunch outbreak in Japan that sickened 2,697 children. In 1997 an EAEC diarrheal epidemic occurred in a village in India and sickened approximately 15% of the population (587). More recent surveillance for EAEC has detected diarrheagenic EAEC strains in Mali (588), Libya (589), sub-Saharan Africa (7), and Nigeria (16), establishing EAEC's presence on the African continent.

Hybrid strains of EAEC and STEC are also becoming recognized. A notable 2011 outbreak in Germany sickened 4,321 previously healthy individuals from 16 countries, where over 900 patients developed HUS and there were more than 50 deaths (590). This Stx-encoding strain was identified as *E. coli* O104:H4, a hybrid pathogen that carried virulence genes found in both typical EAEC strains (*aggA*, *aggR*, *set1*, *pic*, and *aap*) and STEC (stx_2) (45, 46, 330, 585, 591). The *stx*-carrying phage was shown to be most closely related to the phage of an STEC O111:NM strain (592) and was probably acquired quite recently in the phylogenetic history of the outbreak strain. EAEC has an established pattern of acquiring Shiga toxins, as reports from Japan (42), France (43), Northern Ireland (593), and Central African Republic (594) describe *stx*-positive EAEC isolates from patients with HUS. These cases indicate the possibility of outbreaks in the future.

Transmission and reservoirs. Transmission of traveler's diarrhea, which is often caused by EAEC, occurs mostly through contaminated water and food, such as salads (595) (Fig. 5), with desserts and salsa being common contaminated food sources of EAEC in Mexico (596–598). Additionally, food handlers may be carriers of EAEC, emphasizing how important sanitary food handling practices are in prevention of EAEC transmission (599, 600). Remarkably, individuals with an AA rather than an AT or TT genotype at the −251 position of the interleukin-8 (IL-8) gene generated greater fecal IL-8 in response to EAEC and were associated with increased infection with EAEC (601). While atypical EAEC has also been identified in calves, piglets, and horses, animals are not an important reservoir of human-pathogenic typical EAEC (602). However, because EAEC isolates are so heterogeneous, it is premature to rule out animal reservoirs for undercharacterized lineages of EAEC.

Stx-containing strains. During the 2011 European outbreak of 4,321 cases, fenugreek sprouts were identified as the most likely source of infection (603). The seeds were imported as a lot in late 2009 from Egypt, and it is still unknown if the point of contamination occurred at the site where seeds were produced, during transportation, or at the importer (603). By PCR screening of 1,468 French cattle, it was determined that cattle were not a reservoir for the 2011 European outbreak of STEC O104:H4, despite the observation that domestic ruminants are natural reservoirs of STEC (604). Finally, delayed human-to-human transmission did occasionally occur during the STEC O104:H4 infection in Poland, France, Germany, and The Netherlands during the 2011 European outbreak, indicating that while it is rare, humans can act as reservoirs of STEC O104:H4 (44, 605–608).

Pathogenesis

For non-Stx variants, EAEC strain 042 has been used as a prototypical strain to study virulence factors and pathogenicity of diarrheagenic EAEC, as it causes diarrhea in the majority of volunteers (609). However, the encoding genes for numerous adhesins, toxins, and proteins associated with virulence are highly variable among strains (3, 8, 16, 583, 585, 610). Even the site of infection in the gastrointestinal tract is not uniform. For example, the EAEC 042 strain has been isolated from the jejunum in infected volunteers, and in tissue culture it adheres strongly to samples of jejunal, ileal, and colonic mucosa (609, 611). In a controlled study looking at five different non-Stx EAEC isolates from children, each strain had a different affinity for the jejunal, ileal, and colonic mucosae (612). Despite the heterogeneity among the different non-Stx EAEC strains, a general three-part model has emerged for non-Stx EAEC pathogenesis: (i) adherence to the intestinal mucosa, (ii) production of enterotoxins and cytotoxins, and (iii) mucosal inflammation (585).

Adherence. During the first stage of its pathogenesis, adherence to the intestinal mucosa, EAEC aggregative adherence is associated with both fimbrial (aggregative adhesion fimbria [AAF]) and afimbrial adhesins. As reviewed by Estrada-Garcia and Navarro-Garcia in 2012 (585), several different EAEC strains contain different afimbrial adhesins, otherwise known as outer membrane proteins associated with aggregative adherence (585, 613–617) (Fig. 6). Along with the diversity in afimbiral adhesins found among different EAEC strains, there have also been several different AAFs observed by electron microscopy (618–620). Depending upon the EAEC strain, the major structural subunit of AAF has four different variants: AggA (AAF/I), AafA (AAF/II), Agg3A (AAF/III), and Agg4A (AAF/IV) (621–624). These variants are all encoded on the pAA virulence plasmid, which also encodes AggR,

a transcription factor that regulates AAF biogenesis (625, 626). In addition to these AAF variants, other EAEC strains can encode alternative fimbrial structures, such as a type IV pili in EAEC strain C1096 (627, 628). For strains that lack AAFs, aggregative adherence has been linked to the *hraI* gene on the genome (also known as *hek*) or to possession of alternate adhesins such as Hda (629).

Toxin production. For the second stage of EAEC pathogenesis, many different putative virulence factors have been described and reviewed (585, 588, 630–632). The effects of these toxins include microvillus vesiculation, enlarged crypt openings, and increased epithelial cell extrusion (583). These putative toxins include Pet, a cytoskeleton-altering toxin (633), EAST-1, a heat-stable enterotoxin encoded by many other pathogens besides EAEC (634), ShET1, a subunit toxin that appears to induce intestinal cyclic AMP (cAMP)- and cGMP-mediated secretion and is also encoded by *Shigella flexneri* (508), and Pic, a mucinase shared among many different pathogenic *E. coli* and *Shigella* strains (635). Interestingly, the Pet toxin of EAEC belongs to the same protein family as the EspC toxin of EPEC, where the two toxins cleave the actin-binding protein fodrin but have dramatically different mechanisms of entering the host cell (636). Other, uncharacterized toxins identified in genome sequencing and metabolic profiling screens include a potential hemolysin encoded by the *hlyE* gene (27, 577), as well as dispersin, encoded by the *aap* gene (antiaggregation protein, previously named *aspU* [587, 637, 638]). Finally, the EAEC 042 strain also carries the *shF* gene, which is predicted to be similar to IcaB, a mediator of biofilm formation in *Staphylococcus epidermidis* (590, 637, 639). It is known that the AAFs also mediate biofilm formation in EAEC (585, 622, 640). Characterization of toxins encoded by different EAEC strains remains an ongoing and active field of research. AggR is involved in the regulation of many of the virulence genes involved in both the aggregation and toxin production stages of EAEC pathogenesis (45, 46, 330, 625). AggR is a positive regulator and belongs to the AraC family of regulators. It is negatively regulated by the global regulator H-NS and positively regulated by itself and the DNA-binding protein FIS (43, 641).

Inflammatory diarrhea. During the final stage of the EAEC pathogenesis model, there are multiple factors that influence the scale of inflammation, including the host innate immune system and the strain of EAEC (585, 597). During infection of the gastrointestinal tract, EAEC adherence to intestinal epithelial cells stimulates the release of IL-8 and CCL20, which recruits neutrophils and stimulates inflammatory diarrhea (599, 600, 642, 643). Proteomics studies with the EAEC-T8 strain (644) have shown that it interacts with Gp96, a cell surface protein that has significant homology with Hsp90, a protein which triggers signaling pathways in response to binding by other pathogens and leads to activation of inflammatory/immune responses through NF-κB and MAPK (601, 644, 645). In the same proteomics study (644), it was also found that the EAEC-T8 strain interacts with TSP1, an ECM glycoprotein that is rapidly secreted at high levels in inflamed and damaged tissues and has been implicated as a molecular bridge between Gram-positive pathogens and host tissue cells (603, 644). Additionally, AAF/II can elicit a basolateral release of IL-8 from polarized monolayers of T84 human colonic epithelial cells (603, 646). Furthermore, AAFs mediate EAEC-induced polymorphonuclear neutrophil (PMN) infiltration both *in vitro* and *in vivo* by triggering host cells to release an eicosanoid-based PMN chemoattractant generated through 12/15 lipoxygenase (12/15-LOX) activity (647). The released eicosanoid guides PMNs across the intestinal epithelium to the luminal surface by forming a chemotactic gradient (647–649). Finally, the presence of many of the putative virulence factors outlined above correlates with elevated levels of fecal cytokines, interleukins (IL-1ra, IL-1β, and IL-8), alpha interferon (IFN-α), lactoferrin, fecal leukocytes, and occult blood (as reviewed in references 585 and 609).

Clinical Considerations

Symptoms. Diarrheagenic EAEC presents with watery secretory diarrhea, often with mucus, and can be accompanied by a low-grade fever, nausea, vomiting, abdominal pain, and occasionally bloody stool (as reviewed in references 3, 8, 583, 585, 597, 610, 650, 651, and 652). In both developing and developed countries, EAEC affects both adults and children (609, 611, 632, 653–655). It also causes acute and persistent diarrhea in HIV-infected patients (612, 656, 657) and persistent diarrhea in children, which leads to growth and intellectual shortfalls (as reviewed in references 585 and 658). The most significant public health concern stemming from EAEC infections is malnourishment in children in developing countries, as persistent EAEC infections lead to chronic inflammation, which damages the intestinal epithelium and reduces its ability to absorb nutrients (585, 618–620, 658–660). Additionally, the odds of developing postinfectious irritable bowel syndrome are dramatically increased after acute infectious gastroenteritis (661), with some studies reporting EAEC, among other enteropathogens, as a causative agent (662).

Detection. The gold standard method to identify EAEC is to culture five colonies per patient in static Luria broth at 37°C and then infect semiconfluent HEp-2 cells for 3 h and look for the aggregative adherence (AA) pattern (Fig. 7). When treated this way, EAEC aggregates and produces a hallmark "stacked-brick" appearance, where the bacilli are elongated and sometimes line up in a single layer on the surface of the cell (574, 627, 628). However, this method does not distinguish between pathogenic and nonpathogenic strains and is unsuitable for surveillance for EAEC outbreaks, as it requires specialized equipment and is labor-intensive. In a meta-analysis of four different studies examining the distribution of EAEC genes in children who were excreting EAEC, the *aafA*, *astA*, and *4A18* genes were all associated with acute diarrheal illness among children from developing regions. However, the EAEC strains in all of these 4 studies were heterogeneous, with the detection of these genes in each study quite different (632). Additionally, in the same meta-analysis, a DNA probe commonly used for EAEC, which is based upon a 1-kb DNA fragment from the 60-MDa plasmid of EAEC strain 17-2, showed a wide range in sensitivity (15% to 89%), reflective of the heterogeneity among EAEC strains and the lack of defined virulence properties in many EAEC strains (632). Using molecular biology assays such as PCR to identify hallmark virulence genes is problematic, as not all EAEC strains that have the AA binding pattern are diarrheal, and in those that are, there is no single gene or set of genes that distinguishes between pathogenic and nonpathogenic EAEC strains (585).

Historically, the *aggR* gene was thought to be a promising candidate for molecular detection, as it encodes a positive regulator for many virulence factors involved in adherence and toxin production by typical EAEC strains and is carried by more than 70% of EAEC isolates from human patients (583, 663–665). However,

nondiarrheal EAEC strains carrying the *aggR* gene have also been isolated from healthy patients, and detection of this gene in a bacterial isolate from a diseased patient cannot be considered conclusive that it is the causative agent of diarrhea (586, 633, 663–665). Nonetheless, multiple PCR-based assays have been developed to identify the *aggR* gene, and detection of additional virulence genes, such as *aap*, *astA*, and *set1A*, significantly increases the detection of strains associated with causing diarrhea in U.S. and European patients (634, 666, 667). However, strains that do not carry the *aggR* gene have also been isolated from gastrointestinal outbreaks (627).

Currently, amplification by multiplex PCR of either the plasmid-carried gene *aatA* or the chromosomally carried *aaiC* locus is considered sufficient to confirm EAEC in the recently released GEMS, an initiative to comprehensively identify major enteric pathogens rapidly at sites where the diarrheal burden is high (535). *aatA* encodes an outer membrane protein that is part of a protein transporter system (581, 668). In a 2009 study of 252 *E. coli* isolates from children with diarrhea, *aatA* was amplified only in EAEC strains and not in other diarrheagenic *E. coli* strains (669). The other gene used in GEMS, *aaiC*, encodes a protein secreted by the prototypical EAEC strain 042, with AaiC secretion regulated by AggR. *aaiC* was detected in 26 EAEC strains in a study of 35 EAEC strains from geographically distinct regions, indicating that it may be found in high prevalence in EAEC (625). However, amplification of either of these genes does not conclusively distinguish diarrheagenic EAEC from nondiarrheagenic strains (631). In a subsequent study of all EAEC strains that were positive for either *aaiC* or *aatA*, one set of genes in combination, *aaiC* and *agg3/4C* but lacking *agg4A* and *orf61*, was associated with diarrhea cases, and another one, *orf61* in the absence of *pet* and *aafA*, was correlated with control children (631). Additionally, as reviewed in reference 585, in Brazilian children with diarrhea compared to those without, the presence of the *kps* (capsule), *hly*, *aero* (aerobactin), and *aggA* genes was strongly associated with disease (586), suggesting that development of an assay to detect these genes may be a promising method for detection of EAEC strains that cause disease in children in non-European and non-U.S. locations. While current studies to identify conclusive markers for diarrheagenic EAEC strains are promising, identification of molecular markers for rapid detection will remain a challenge due to the heterogeneity of EAEC (585).

Treatment. (i) Treatment of traveler's diarrhea. Antibiotics are generally recommended for treatment of traveler's diarrhea, but because EAEC is increasingly resistant to various antibiotics, selection of the appropriate antibiotic should take into account the region of the world where the infection was acquired, as there are different antimicrobial susceptibility patterns for each geographical region (670–672). Like *Shigella* infections, EAEC infections are often successfully treated with ciprofloxacin and other fluoroquinolones, but there are multiple antibiotic-resistant strains (597, 673). For example, in southern India, EAEC is increasingly resistant to quinolones (672). In adult patients in the United States, EAEC is susceptible to rifaximin or a single dose of azithromycin with or without loperamide (670, 674, 675). Elimination of persistent diarrhea may be problematic, as internalized EAEC was observed in intestinal epithelial cells *in vitro*, suggesting that EAEC may protect against host clearance by intracellular persistence (676).

(ii) Treatment of infections caused by Stx-containing EAEC strains. Prior to the 2011 outbreak in northern Germany, there was no standardized treatment for Stx-containing EAEC. During the 2011 German outbreak, 3 children with Stx-associated HUS showed rapid clinical improvement with eculizumab, but results from a subsequent nonrandomized trial with 298 patients were unclear (677–679). Patients who had no clinical improvement during plasmapheresis and/or were suffering from severe neurological complications were preferentially selected for the trial, leading to a selection bias that complicates the results (677, 679). As eculizumab disrupts the complement cascade, clinicians at the time were required to treat with a prophylactic antibiotic to prevent meningitis. In general, antibiotics are normally not recommended for STEC, as they increases the risk for development of HUS by stimulating Stx production. Because of this risk, clinicians treating *stx*-expressing EAEC strain O104:H4 selected azithromycin, which *in vitro* represses *stx* expression (677, 679, 680). Monitoring of STEC shedding in patients receiving azithromycin showed that these patients were rapidly decolonized (677, 681). Because of this, long-term (>28 days) carriers of STEC O104:H4 were treated with azithromycin, and after a 3-day course of treatment, all 15 were negative for shedding as well as HUS-related symptoms (677, 681). Further studies have since shown that subinhibitory concentrations of ciprofloxacin increase Stx production in STEC O104:H4 but that meropenem, rifaximin, tigecycline, and azithromycin do not (677, 680). Stx production by STEC O157:H7 responds differently to these same antibiotics (677, 680). Nonetheless, the use of azithromycin to eliminate Shiga toxin-containing strains such as O104:H4 from patients is still considered a controversial treatment; if used early in treatment, it is still unclear if it plays a role in the development of HUS (682), and if used later in treatment, it may actually increase the risk of sudden cardiac death (683).

(iii) Treatment of immunocompromised patients. In a study of HIV-infected children in Peru, 74% of the diarrheagenic *E. coli* strains detected were highly resistant to ampicillin (74%) and cotrimoxazole (70%) (684). The current Infectious Disease Society of America practice guidelines for the management of infectious diarrhea recommend fluoroquinolones for the treatment of immunocompromised patients (685). AIDS patients with EAEC-induced diarrhea have been successfully treated with ciprofloxacin, with fecal shedding eliminated and 79% of total symptoms improved or eliminated (686).

Vaccines and other preventative treatments. As EAEC proteins are antigenic, it remains possible that a vaccine could be developed, but as of yet, there is none. As reviewed in reference 670, in a vaccine study using the ETEC heat-labile toxin, the rate of infection and severity of disease caused by ETEC was decreased, and despite the presence of EAEC in the placebo groups, the vaccine-treated group had no EAEC detected, suggesting that the vaccine may also exert protection against EAEC (687). *In vitro* treatment with lactoferrin inhibits EAEC enteroadhesion and biofilm formation, making it a potential but untested nonantibiotic treatment for the prevention of EAEC (670, 688).

ENTEROTOXIGENIC *E. COLI*

ETEC is a diverse pathotype that is a major cause of traveler's diarrhea and endemic in most underdeveloped countries (30). In these developing countries, ETEC can be isolated from both symptomatic and asymptomatic carriers, with significant mortality rates in children (30, 689). ETEC is defined by its ability to produce either a heat-labile (LT) or a heat-stable (ST) entero-

toxin, and it carries a diverse set of colonization factors (CFs) for adherence to the intestinal epithelium. In addition to the impact of ETEC on humans, the swine industry is also adversely affected, as ETEC-induced diarrhea in neonatal and postweaning piglets causes much morbidity and mortality. This review of ETEC will focus on its human-related illnesses, building on recent knowledge from the excellent reviews by Nataro and Kaper and by Qadri et al. (8, 30).

Classification

Common serotypes. The distribution and occurrence of O and H antigens across various regions were thoroughly reviewed by Wolf in 1997 (690). There are at least 78 detectable O antigens and 34 H antigens associated with ETEC. Among the most common serotypes were O6:H16 (LT/ST), O8:H9 (ST only), O25:NM (LT only), O78:H12 (ST only), O148:H28 (ST only), and O153:H45 (ST only); however, their prevalence and phenotypes (e.g., which toxins they harbor or CFs they express) can vary depending on the location (690). Many of these serotypes were also commonly isolated from surface water in Bangladesh (691) and from outbreaks in the United States (692) and Japan (693). More recently, O169:H41 (ST only) has become an emerging serotype that has been involved in several outbreaks in the United States, where it was rarely seen before 1996 (694, 695).

Lineages. MLST of ETEC isolates groups them across all phylogroups (696). Further studies on a set of 1,019 human isolates found that they could be grouped into 42 distinct clonal groups, with diverse toxin and CF profiles spread across all lineages (18).

Epidemiology

While ETEC is a major cause of traveler's diarrhea, there is significant morbidity and mortality associated with ETEC-induced diarrhea in children in developing countries. Cohort studies following children in Argentina, Bangladesh, Egypt, and Guinea-Bissau have looked at the distribution of toxins and CFs and their frequency and contribution in diarrheal disease (697–700). In these studies, ST-positive ETEC strains were commonly associated with diarrhea. The GEMS case-control study also reported ST-positive ETEC, but not LT-only-positive ETEC, as a major contributor to infantile diarrhea (7). ST-positive ETEC strains were also found to be associated with an increased risk of death in children.

Incidence. A review of population-based studies estimates that there are nearly 840 million annual cases of ETEC in developing countries, with approximately 280 million of these in children aged 0 to 4 (701). Children under the age of 4 can experience up to 0.47 to 0.54 ETEC episode/person/year, which drops to 0.11 to 0.15 ETEC episode/person/year after the age of 5 (701), possibly due to immunity acquired from prior infections (702). A review of population studies in developing countries showed that ETEC was the etiological agent isolated in a median of 13% of diarrheal cases in children (689). The authors of that review also estimated that 325,000 children under the age of 5 die each year due to ETEC-associated illness (689). An additional 46.6 million children under the age of 4 are thought to be asymptomatic carriers of ETEC (701). In rural Egypt, a study showed that 84% of children under the age of 3 will have at least one ETEC-related episode of diarrhea (703). The authors estimated that there were approximately 1.5 episodes/child/year related to ETEC.

In developed countries, ETEC is not routinely tested for in patients presenting with diarrhea (704); therefore, incidences of ETEC illness and epidemiological studies in these areas are lacking. However, travelers to high-risk, developing regions such as Latin America, Africa, and certain regions of Asia are at risk of developing traveler's diarrhea. Despite a decrease in overall ETEC-related traveler's diarrhea cases in Latin America and Africa, ETEC is still the most common etiological agent detected and makes up approximately 30% of these cases (462).

Adhesin and toxin profiles of ETEC isolates are also variable and differ among different geographical regions and populations. A systematic review of the literature across 35 countries found overall that approximately 45% of ETEC isolates expressed ST only, 27% expressed LT only, and 33% expressed both ST and LT (705). Furthermore, the authors also determined the regional prevalence of colonization factors and showed that CFs such as CFA/I, CS6, and CS21, were among the most common CFs detected in ETEC isolates worldwide (705).

Host factors. A study in Bangladesh, following ETEC diarrhea of children during their first 2 years of life, noted that ST-expressing ETEC more frequently infected children with blood type A or AB than children with blood group O (698). Further studies determined that children with Lewis antigen Le(a+b−), but not Le(a−b+), were more susceptible to illness caused by ETEC that expresses the CFA/I group of CFs (706). This association was suggested to be because of the ability of CFA/I to adhere to Le^a-terminated glycosphingolipids but not Le^b-terminated glycosphingolipids on host cells (706, 707).

Transmission, reservoirs, and sources. ETEC infections are transmitted through the fecal-oral route. Exposure to ETEC is usually from contaminated food and drinking water (Fig. 5). Some examples of high-risk foods contaminated with etiological agents for traveler's diarrhea include food that is left at room temperature, table-top sauces, certain fruits, and food from street vendors (reviewed in references 708 and 709). Additionally, surface water in developing regions such as Bangladesh can also contain these organisms and may serve as an important source of infection during contact with this water (691). Foods can be contaminated by infected food handlers (710), by asymptomatic carriers (711), or when vegetable crops are irrigated with untreated water (712). The persistence and ability of ETEC to survive in these environments are mostly unknown. One study showed that ETEC was able to survive for up to 3 months in freshwater (713) and was able to form biofilms in drinking water sources (714). The infectious dose, compared to that of other *E. coli* pathotypes, is relatively high and is thought to be between 10^6 and 10^8 organisms (715). However, this infectious dose is based on laboratory-grown cultures and may differ from the actual dose from natural transmission. One investigation of an outbreak in a Japanese prison estimated that the infectious dose in contaminated pickles was between 25 and 1,000 organisms (716).

Outbreaks. Outbreaks in developed countries are sporadic and can consist of more than one serotype (692). These outbreaks are likely underdetected, as ETEC is rarely sought in diarrheal cases (704). A study of the CDC's ETEC outbreak investigations from 1996 to 2003 identified 3 outbreaks related to international cruise ships, mainly linked to drinking water, and 13 domestic outbreaks (694). This study identified serotype O169:H41 (ST only) as the most common isolate, which is considered an emerging strain in the United States (694) and which affected over 30 people in an outbreak in Tennessee (695). The largest ETEC outbreak reported in the United States was from the state of Illinois in 1998, where

serotype O6:H16 (LT/ST) was isolated from several patients. Although only 120 cases were documented, it was estimated that 3,300 people may have become ill due to food prepared by a delicatessen that catered several events over the span of a few days (717). Further outbreaks were reported from a sushi restaurant in Nevada (710) and a buffet-style lunch in Illinois (718).

Outbreaks have occurred in other developed countries outside the United States. In 2006 a mixed outbreak of ETEC and *Salmonella*, possibly linked to pesto, caused 217 illnesses in a Danish school (719). Denmark had an additional 5 outbreaks of ETEC mixed with norovirus in early 2010, which were linked to contaminated lettuce (720). Additionally, Japan has had 131 ETEC-related outbreaks from 1966 to 2009 that originated from various contaminated food and water sources (693), while Israel had a large outbreak of ETEC, likely due to improperly sanitized water, that affected 175 military personnel and at least 54 civilians (721).

Although ETEC is endemic in most developing countries, epidemic outbreaks have occurred during major floods in Bangladesh, where ETEC was identified in 18% of diarrheal cases studied, second only to *Vibrio cholerae* (722). In hospitals, outbreaks have been reported in neonatal intensive care units, likely due to contaminated milk prepared by an asymptomatic carrier (711).

Pathogenesis

Analysis of ETEC genomes has shown that there may be a core set of genes shared between ETEC isolates; however, no virulence factors were attributed to these core sequences (24). Indeed, it has been suggested that the acquisition of plasmid-borne toxins and virulence factors may be the major driving force that makes ETEC a pathogen (696, 723). ETEC causes disease by colonizing the small bowel through attachment to the host epithelial lining by surface proteins called CFs and possibly other surface structures (Fig. 6). Adherent ETEC then elaborates enterotoxins that cause the clinical manifestations typical of ETEC-induced diarrhea. Unlike for most other pathotypes, almost all known virulence factors are encoded exclusively on plasmids. With more whole genomes being sequenced, it is possible that chromosomal virulence factors will be identified in the future.

Adhesion. ETEC CFs are named coli surface antigens (CS), suffixed with a number, except for CFA/I. They are surface structures that are required for colonization and disease. Currently, at least 22 CFs have been characterized; however, between 30 and 50% of ETEC isolates have undetectable CFs (30). This suggests that there are still unknown CFs or that they are undetectable under typical laboratory conditions. The advancement of whole-genome sequencing could identify potential new CFs. The most recently identified CF, CS23, is an afimbrial adhesin (724).

These surface structures are either fimbrial, afimbrial, helical, or fibrillar and are encoded mostly on virulence plasmids (30, 725). The molecular mechanisms of their assembly and structure have been reviewed elsewhere (726). CFs are subdivided into different families, with CFA/I, CFA/II, and CFA/IV being the most common, depending on the region (30, 725).

CFs bind to different receptors on host cells. For example, CS6 was found to bind fibronectin (727) and sulfatide (SO_3-3Galβ1 ceramide), a major glycosphingolipid (728), while sequence variation changes binding affinity to host cells (729). CFA/I was also found to bind to glycoproteins and glycosphingolipids found on the surface of host cells in the small intestine (707). CFs can also function beyond host cell adherence; longus (CS21) has been shown to be involved in twitching motility (730) and self-aggregation, which was shown to be protective against antimicrobials, *in vitro* (731).

Other adhesive proteins found in some ETEC isolates are encoded in the *tia* and *tib* loci. Based on a Chilean study, *tia* and *tib* were found in 9% and 11% of isolates, respectively (732). Genes from the *tia* locus (also found in strains of EPEC, EAEC, and *S. sonnei*) have been shown to be involved in invasion of and adherence to epithelial cells (733, 734) and interact with heparan sulfate proteoglycans (735). The *tib* locus is also involved in adherence and invasion (736, 737). More recent studies have demonstrated the involvement of TibA in autoaggregation and biofilm formation (738, 739). Finally, a protein, EtpA, was found to contribute to adherence to epithelial cells and is thought to act by being transiently exposed at the tip of the flagellum to mediate attachment (740, 741). EtpA is found in approximately 75% of ETEC isolates, based on work by Del Canto et al. (732).

ST. Harboring a heat-stable enterotoxin (ST) is a characteristic that can define an ETEC strain. Two STa (or ST-I) variants have been found in human disease, STp and STh (8), and will be referred to here as ST. STb (or ST-II) can be found in strains that colonize animals. These toxins can be found as the only enterotoxin (ST only) or in combination with LT (LT/ST). Overall, ST is found in about 75 to 80% of ETEC isolates (approximately 45% ST only and 33% LT/ST) (705) and is found more frequently in severe human disease than LT-only isolates (30). STs are small enterotoxins in comparison to LT and are generally 18 or 19 amino acids in length, following processing and secretion from the bacterial cell.

The molecular mechanisms of ST are well described in the literature (for a review, see reference 742), but the dogma on secretion has been challenged (reviewed in reference 743). Generally, these small peptides mimic the hormone guanylin and thus bind guanylyl cyclase C (GC-C) receptors on the intestinal epithelium. This overactivation of GC-C, caused by ST binding, leads to an accumulation of cGMP, which indirectly promotes cystic fibrosis transmembrane receptor (CFTR) to secrete chloride into the intestinal lumen and also indirectly inhibits the sodium-hydrogen exchanger, preventing absorption. Although ST-induced chloride and fluid secretion into the lumen is widely regarded as the cause of watery diarrhea, an *in vivo* study using volume recovery in the rat jejunum suggested that diarrhea caused by ST may instead be due to impaired fluid absorption (744; recently discussed in reference 743).

LT. Heat-labile enterotoxin (LT) is the second enterotoxin that can define ETEC. There are two classes of LTs, the prototypical LT-I (usually referred to as LT, which will be followed in this review) and LT-II, which has differences in its B subunit receptor affinity and immune properties (reviewed in reference 745). LT-II can also be subcategorized into LT-IIa, LT-IIb, and the more recently discovered LT-IIc (746). Generally, LT-I is more consequential in human illness, but LT-II has been isolated from human cases of ETEC diarrhea (747). While LT is found encoded on virulence plasmids, all LT-II variants have been found encoded in the chromosome and may have been acquired by a phage (747).

Assembled as an AB_5 toxin, LT is a large toxin that is about 80% identical to the cholera toxin from *V. cholerae*. As with ST, the molecular mechanisms of LT are well studied and extensively reviewed (748). Briefly, the pentameric B subunits bind to GM1 gangliosides at lipid rafts to deliver the catalytic A subunit inside

the cell. Once inside the cell, the A subunit is trafficked through the endoplasmic reticulum (retrograde) and delivered to the host cytoplasm, where it ADP-ribosylates $G_{s\alpha}$, inhibiting its GTPase activity and increasing cyclic AMP levels. Increased cAMP opens the CFTR, resulting in electrolytes and fluid loss into the intestinal lumen. Additionally, fluid loss into the intestinal lumen may also be a consequence of intestinal barrier disruption caused by LT (749). As discussed in reference 748, LT has also been shown to interact with lipid A of LPS (association with OMVs), blood type A sugars, and non-GM1 gangliosides, albeit at lower affinities.

Additional roles have been characterized for LT, including contributing to adherence to host cells through activation of host signaling pathways (750, 751), as well as suppression of antimicrobial peptide expression (752).

EAST1. Very little is known about the involvement of EAST1 in disease, but it is carried by many pathogenic and commensal *E. coli* strains (148), leading to uncertainty about EAST1 involvement in diarrhea. A recent study showed that EAST1 in ETEC may not be involved in diarrhea (753).

Other virulence factors. The SPATE EatA is a 104-kDa plasmid-encoded protein that contributes to increased virulence in a rabbit ileal loop model (754). More recent work has shown that EatA degrades EtpA and contributes to LT delivery, as an *eatA* mutant was hyperadherent to epithelial cells but was unable to efficiently deliver LT (755). Del Canto and colleagues identified EatA in approximately 70% of Chilean ETEC isolates and suggested that it could be suitable as a marker for epidemiological studies (732).

A small, 12.6-kDa secreted protein, called CexE, was identified to be regulated by a known virulence regulator (756) and has been suggested to function similarly to dispersin from EAEC (723).

Clinical Considerations

Symptoms and onset of disease. ETEC causes mild to severe watery diarrhea (usually nonbloody) that has a clinical presentation very similar to that of cholera and can rapidly lead to dehydration (30). Illness is sometimes accompanied by headaches, fever, abdominal cramping, nausea, and vomiting (30, 692, 694). The onset of ETEC-related diarrhea is generally quick, with an incubation period that can be as short as 5 h but generally averages between 1 and 2 days following ingestion (692, 694). The duration of diarrhea is usually about 3 to 5 days (692, 694) but can be prolonged for more than a week (718). Mortality rates with proper treatment are very low (<1%) (30).

Complications. ETEC diarrheal illness in children in developing countries under the age of 2 has implications for growth. A study following children in Bangladesh found that children who experienced an episode of ETEC diarrhea were more malnourished and growth stunted than those who had non-ETEC diarrhea (698). There is also a suggestion in the literature that people who have experienced traveler's diarrhea have a 5-fold-increased risk of developing postinfectious irritable bowel syndrome (757). Although ETEC is commonly implicated in traveler's diarrhea, the etiological agent responsible for this increased risk of irritable bowel syndrome is unclear (662).

Detection. ETEC is defined by the presence of either enterotoxin, and thus identification is made by detection of LT, ST, or both. As with most other *E. coli* pathotypes, ETEC cannot be readily distinguished on typical MacConkey media from commensal *E. coli*, and thus molecular techniques must be used. The history of ETEC detection with methods such as monosialoganglioside GM1 enzyme-linked immunosorbent assay (ELISA) for LT and ST, multiplex PCR for toxins and CFs, and monoclonal anti-CF antibodies has been reviewed by Qadri and colleagues (30). A recent paper highlighted the importance of screening multiple colonies for ETEC, as testing only 5 colonies missed approximately 50% of ETEC-positive stools identified by screening 20 colonies (758). Additionally, toxin-encoding genes may be lost during handling, as one study showed that toxin genes became undetectable in 119 of 1,197 isolates (702).

A comparison of the phenotypic and genotypic ETEC detection methods suggested that PCR methods for enterotoxin detection were highly specific and sensitive, and CFs could also be detected by PCR or dot blots using monoclonal antibodies (759). PCR-based approaches are particularly useful in studies where monoclonal antibodies to enterotoxins or CFs are unavailable. A multiplex assay that was able to detect 19 different ETEC virulence factors was developed (760). Additional studies have shown that fecal smears on hemocult cards allowed for long-term storage and isolation of ETEC DNA, which could be useful in epidemiological studies when standard methods for culture are not readily available (761).

Treatment. ETEC-associated diarrhea is self-limiting, and in general, oral rehydration and maintaining fluid and electrolyte balance through diet are very effective for both children and adults (8, 30). In severe cases of dehydration, intravenous rehydration may be necessary (691). Antisecretory drugs, such as loperamide, may be beneficial in reducing the number of stools in adults and are usually safe to use in combination with antimicrobials (reviewed in reference 762). Antibiotics such as fluoroquinolones, azithromycin, and rifamixin can lessen the duration of infection and are commonly used in self-treatment of traveler's diarrhea. However, the decision to use antibiotics has to be weighed against possible adverse effects, such as the selection of antibiotic-resistant bacteria and the possibility of increasing the predisposition to developing other intestinal infections (762). Recommendations for preventative measures for traveler's diarrhea, such as chemoprophylaxis, immunoprophylaxis, and food safety, have been discussed elsewhere (763).

Antimicrobial resistance. A study looking at the changing antibiotic resistance profiles of ETEC showed that nearly 60% of isolates were resistant to trimethoprim-sulfamethoxazole and tetracycline and approximately 50% to ampicillin for isolates from 2001 to 2004 (764). Of concern is the noted increase of ciprofloxacin resistance from 1% in the period of 1994 to 1997 to 8% in 2001 to 2004, where most of the ciprofloxacin resistant ETEC strains were isolated from patients who traveled to India. Ciprofloxacin-resistant isolates were also recovered from travelers from North Africa and Southeast Asia. This trend of emerging antibiotic resistance in ETEC was reviewed by Qadri et al. (30). In addition, it has been shown that ETEC rifaximin-resistant mutants can be selected *in vitro* and might occur more frequently than ciprofloxacin-resistant mutants (765).

Vaccines. Immunity against ETEC reinfections has been observed in adult volunteers and naturally in children (702, 715) and supports the concept that a successful ETEC vaccine may be possible. Unfortunately, the diversity of ETEC strains has made vaccine development challenging; there are too many serotypes to target the O antigen, and ETEC has a virulence profile that differs

between isolates, depending on geography (i.e., CF and toxin distribution).

Vaccines that are currently being evaluated can be grouped into different types, such as toxin-based, live attenuated, inactivated whole-cell, hybrid, and fimbrial antigen vaccines (766). LT is strongly immunogenic and cross-reacts with the B subunit of cholera toxin (CTB). The recombinant CTB (rCTB) has been used in an inactivated whole-cell oral vaccine (Dukoral), where it provides short-term protection against ETEC in travelers (as discussed in reference 709), as well as in the live attenuated cholera vaccine Peru-15pCTB, where sera from mice and rabbits could neutralize LT toxins (767). A transdermal patch made up of LT was shown to have few adverse effects and to elicit an immune response to the toxin (768, 769). A phase II study showed that the transdermal patch reduced the occurrences of traveler's diarrhea in travelers to Mexico and Guatemala compared to that in placebo-controlled travelers (687). Expression of LTB in the *Salmonella enterica* serovar Typhi live attenuated vaccine has been shown to elicit antibodies to LT in mice and in approximately 70% of inoculated human volunteers (770, 771). Additionally, LTB expressed in potatoes and corn and then ingested by human volunteers was able to cause development of IgG antibodies to LT in most volunteers (772), while rice expressing CTB (MucoRice-CTB) was protective against *V. cholerae* and ETEC challenge in mice, through mucosal IgA (773). Work is also being done to improve the safety of LT. A recent study characterized a new mutant LT called dmLT that is quickly degraded, has reduced toxicity, and may be useful in oral vaccines (774).

LT is present in only approximately 60% of isolates (LT only and ST/LT), while the poorly immunogenic ST is found in about 75 to 80% (705). Despite the poor immune properties, there is still interest in developing ST as a vaccine. In a recent study a hybrid toxoid with ST fused to LT was made, where both proteins were mutated to abate any toxicity, and showed an antibody response to both LT and ST (775, 776). A green fluorescent protein (GFP)-ST-LTB fusion that was expressed in *Lactobacillus reuteri* was given as a live oral vaccine to mice and was able to elicit antibodies to the protein fusion and protect mice against ETEC challenge (777). However, since ST is similar to guanylin, there is concern that there may be cross-reactivity issues with vaccines that target ST and its GCC-C receptor (reviewed in reference 778).

An oral, inactivated vaccine that expressed a mix of CFs and is supplemented with rCTB (rCTB-CF ETEC) has been extensively studied (discussed and reviewed in reference 779). This vaccine was studied in individuals traveling to Mexico and Guatemala, where the severity of diarrhea was milder in vaccinated travelers but the number of diarrheal incidences was not significantly different than that in placebo controls (780). This was further extended to a study in Egyptian children, aged 6 to 18 months, where the vaccine was not protective against diarrhea (779). To improve protection of ETEC vaccines, studies have started to optimize expression of CFs in nonpathogenic *E. coli* hosts. Overexpression of various CFs and hybrid CFs in a murine model showed an immune response to the CFs that was higher than that for reference ETEC strains (781–784). A recent publication described a phase 1 trial in volunteers using an *E. coli* strain overexpressing CFA/I coupled with a hybrid LT and CT (LCTBA) and demonstrated that it was safe and elicited an antibody response to both LTB and CFA/I (785).

Surface antigens may provide an alternate strategy for vaccine development. Recently, a study of chromosomally expressed autotransporter genes in ETEC that are not found in commensal K-12 *E. coli* identified two proteins, pAT and Ag43, that are detected using sera from ETEC-infected patients (786). The passenger domains of these proteins were found to be protective in a murine model. Other studies have shown that surface proteins, such as LT, flagellin, EatA, EtpA, CexE, and TibA, could be detected by sera from ETEC-infected mice or humans (787), where it was previously demonstrated that recombinant flagella and EtpA were protective in mice (788). Follow-up studies demonstrated that many of these proteins could be found in OMVs that provide protection against ETEC colonization in mice (789).

Live attenuated vaccines expressing either CFA/I (ACAM2010), CFA/II (PTL-003), or a combination of CS1, CS2, and CS3 (ACAM2017) were first evaluated in phase 1 trials and resulted in an antibody response to their respective CFs (790–792). Follow-up studies with PTL-003-vaccinated human volunteers did not show adequate protection against ETEC-induced diarrhea (793). This idea of a live attenuated vaccine was further developed by engineering three strains that, collectively, covered 6 CFs as well as LTB when mixed together to form ACE527 (794). Phase 1 studies with ACE527 demonstrated safe use in human volunteers and immune responses to all CFs and LT (795). A recent phase 2 study showed a 27% reduction in the incidence of moderate to severe diarrhea compared to that in placebo controls following ETEC challenge, but it was not significant. However, there were improved outcomes noted from secondary endpoints (796).

The expression and delivery of ETEC antigens has been attempted in other bacterial hosts. For example, *Bifidobacterium infantis* strains were recently used to express CFA/I or LTB, and these strains together conferred the most protection to rats following challenge with ETEC (797). Other bacteria, such as *Shigella* spp. (798, 799), *V. cholerae* (781), and STEC O157:H7 (800) have also been used to deliver ETEC antigens to experimental animals.

DIFFUSELY ADHERENT *E. COLI*

The diffusely adherent *E. coli* (DAEC) pathotype describes diarrheagenic *E. coli* strains that attach to cells but do not fall into classical patterns of adherence, such as localized or A/E (8). They have now emerged as a unique group and are considered distinct from other pathotypes, but because of difficulties in classification and identification, the designation of DAEC as a distinct enteric *E. coli* pathotype (801) requires further epidemiological studies.

Classification

DAEC has been classically defined by its diffuse adherence (DA) to cultured epithelial HEp-2 cells, where bacterial adherence occurs over the entire surface of the cell in a scattered pattern (Fig. 7) (86). In a 2005 study of 112 DAEC isolates from diarrheagenic Brazilian patients and controls, 45 total serotypes were found, with 19 of them unique to patients with diarrhea (802).

Epidemiology

Incidence, outbreaks, transmission, and reservoirs. As mentioned above, detection methods for diarrheagenic strains of DAEC are still being developed, and currently there is no universal method to detect strains in a clinical setting that are responsible for diarrhea. Thus, the epidemiology of diarrheagenic DAEC remains unclear. DAEC isolates have been identified from children

with diarrhea in Chile (75), Mexico (803), Australia (804), the United Kingdom (805), Brazil (802), Peru (806), Columbia (807), and many other countries, including the United States. *E. coli* isolates from 200 children in Brazil who had diarrhea revealed DAEC in 23% of 1,801 isolates, further suggesting it is a widespread pathotype that causes diarrhea (808). However, in some of these studies DAEC was also detected in healthy age-matched controls who did not present with diarrhea at rates similar to those for DAEC detection in ill patients, underlining the importance of developing a method to rapidly and conclusively identify DAEC strains that are causative for diarrhea. It is unknown how DAEC is transmitted or its reservoir.

Pathogenesis

As extensively reviewed by Le Bouguénec and Servin in 2006 and by Servin in 2005, the prototypical strain C1845 is a DAEC strain that encodes Afa/Dr adhesins. The Afa/Dr adhesins are a class of adhesins that includes the AfaE-I, AfaE-II, AfaE-III, AfaE-V, Dr, Dr-II, F1845, and NFA-I adhesins (809, 810). In Afa/Dr DAEC, the Dr and the F1845 adhesins bind to brush border-associated decay-accelerating factor (DAF), a molecule that is highly expressed on the apical surface of polarized epithelial cells. After binding, cytoskeleton rearrangement is induced, destroying or partially rearranging microvilli (809–814) (Fig. 6). Some Afa/Dr DAEC strains also bind the human carcinoembryonic antigen-related cell adhesion molecule (CAECAM) family of receptors in a process that leads to internalization into undifferentiated epithelial cells (815). The internalization mechanism is microfilament independent, microtubule dependent, and lipid raft dependent, and DAEC survival is better for bacteria internalized in the vacuoles of cells expressing DAF (809, 816). In addition to binding DAF and the CAECAM family of receptors, Afa/Dr DAEC has also been shown to bind type IV collagen through the Dr adhesin, an interaction that is important for urinary tract infections caused by Afa/Dr DAEC. However, it remains uncertain if this interaction plays a role in diarrheal disease, as type IV collagen is never present at the apical domain of polarized epithelial cells, the site of Afa/Dr DAEC colonization (810).

After binding DAF, disassembly of F actin and villin results in brush border lesions. This eventually leads to a loss of microvilli due to defective expression of brush border-associated functional intestinal proteins (811, 817). Rearrangement of the tight-junction-associated proteins ZO-1 and occludin after infection by Afa/Dr DAEC strains leads to increased paracellular permeability but does not affect transepithelial electrical resistance (813).

After binding of Afa/Dr DAEC strains with DAF, the proinflammatory cytokine IL-8 is produced through flagellar stimulation of Toll-like receptor 5 (TLR5), resulting in activation of mitogen-activated protein kinase, extracellular signal-regulated kinases 1 and 2 (ERK1/2), P38, and Jun-C kinase (818–822). After IL-8 activation, PMNs migrate across the epithelial barrier, promoting TNF-α and IL-1β production, which results in upregulation of DAF (820). Some have speculated that because DAF is present at increased levels in Crohn's disease (CD) patients, persistent carriage of Afa/Dr DAEC strains by these patients may contribute to inflammation (809, 822, 823). From the use of probes to detect Afa/Dr DAEC, it is known that this pathotype is present in ileal mucosa isolated from patients with CD (809, 824).

While the pathogenesis of typical Afa/Dr DAEC is beginning to be characterized, much remains to be discovered for atypical diarrheagenic DAEC strains. There are two different subclasses of atypical DAEC. One subclass contains all the adhesins typical of the Afa/Dr family of adhesins in another *E. coli* background, such as diffusely adherent EPEC (810). In the other subclass of atypical DAEC, the bacterium does not bind DAF and expresses a different array of adhesins on its surface, including AfaE-VII, AfaE-VIII, AAF-I, AAF-II, and AAF-III (810). In this subclass of atypical DAEC, IL-8 is still stimulated by DAEC strains that have been internalized by an uncharacterized mechanism, suggesting that it may elicit pathogenesis mechanisms similar to those of typical Afa/Dr DAEC (825).

Clinical Considerations

Symptoms and detection. DAEC is associated with watery diarrhea that can become persistent in young children, with an increase in severity of disease from the age of 18 months to 5 years (3, 810, 826). It is thought that older adults become asymptomatic carriers, and it has been speculated that DAEC carriage may lead to chronic inflammatory intestinal diseases such as CD (809).

DAEC strain C1845 has surface fimbriae (F1845) that are homologous with members of the Afa/Dr family of adhesins. Therefore, in classical hybridization and subsequent PCR-based screens for detection of DAEC strains, probes and PCR primers were developed to detect *daaC*, *daaE*, and *afaB* and *-C*, which are common genes in operons that encode the Afa/Dr family of adhesins (827–830). However, in a study of 40 infants under 1 year old who had diarrhea in Brazil, 10% of the adherent *E. coli* strains detected were diffusely adherent in tissue culture but were not detected with the *daaC* probe (831), indicating that the absence or presence of these genes cannot be considered conclusive in the identification of all DAEC in clinical screens. Additionally, the *daaC* probe cross-reacts with a subset of EAEC strains that cause diarrhea, further confounding the conclusive identification of diarrheagenic DAEC in the field (801). More recent studies have shown that in 61 diarrheagenic patients, only 9.8% of the DAEC isolates hybridized with *daaE*, while 17.6% of healthy controls also contained DAEC strains that hybridized with *daaE* (802). This study revealed that for the DAEC isolates from patients there was wide array of probes that corresponded with virulence, but no single overall pattern was immediately obvious that could be used to universally detect pathogenic DAEC isolates (802). These probes included sequences for hemolysin (*hly*), aerobactin siderophore (*iucA*), yersiniabactin operon (*irp2*), EAST1 toxin (*astA*), *shET1*, cytotoxic necrotizing factor 1 (*cnF1*), outer membrane protein AIDA-I (a 450-bp EcoRI fragment of pIB6), afimbrial adhesion (*afa*), adhesion subunit type 1 fimbriae (*fimH*), aggregative adhesion fimbria type III (*agg-3A*), P fimbria (*pap*), and S fimbria (*sfa*) (802).

Treatment, antibiotic resistance, and vaccines. Rehydration is currently the only treatment recommended for the watery diarrhea caused by DAEC. Among 112 DAEC strains isolated in Brazilian children, 70% of the strains were resistant to multiple antibiotics, and over 50% were resistant to either ampicillin, cephalothin, co-trimoxazole, streptomycin, sulfonamide, or tetracycline (802). Only 20% of the strains were resistant to chloramphenicol, and all DAEC isolates were susceptible to ceftazidime, gentamicin, lomefloxacin, ofloxacin, and nalidixic acid (802). At the date of this publication, no vaccines for any strain of DAEC exist.

ADHERENT INVASIVE *E. COLI*

Adherent invasive *E. coli* (AIEC) has been implicated as one of the causative agents for CD, which is a cause of inflammatory bowel disease (IBD) affecting mainly the small bowel. There is no single causative agent of CD identified, and the current hypothesis is that disease is caused by a combination of factors, including genetics, the intestinal microbiota, environmental factors, and enteric pathogens. A German nationwide study compared CD concordance in twin pairs and showed that 35% of monozygotic pairs were concordant for CD, compared to only 3% of dizygotic pairs (832). Concordance of CD in monozygotic twin pairs provides strong evidence for a genetic predisposition driving disease, while the 65% discordance of disease points to the importance of other environmental factors leading to disease. Current research supports the hypothesis that a breach in the intestinal epithelium, driven by genetic predisposition or largely by as-yet-unidentified environmental factors, occurs, allowing luminal contents and the microbiota to interact with the underlying immune cells of the lamina propria (833). In genetically susceptible individuals (834, 835), this breach is followed by a weak inflammatory response and impaired clearance of the luminal matter, leading to chronic inflammation and development of CD lesions (835).

In addition to microbial dysbiosis and exposure to adverse environmental factors, several enteric pathogens have been implicated as the causative agent of the inflammation in CD patients (836). Pathogens of interest include *Mycobacterium avium* subsp. *paratuberculosis*, *Campylobacter* species, cytomegalovirus, and adherent invasive *E. coli* (AIEC) (836).

Classification

The AIEC pathotype does not express common virulence factors found in various other pathogenic *E. coli* strains and the genetic basis for its proinflammatory and invasive phenotype is not fully understood (837). Currently the virulence-like features associated with AIEC are detectable only phenotypically, but recent genomic studies are beginning to define some unique genetic determinants that could be used for pathotype identification in the future (837). AIEC strains have a high variability of O:H serotypes, with the O6 and O22 serogroups being most prevalent (838). AIEC strains cluster within the B2 phylogenetic group and are most closely related to extraintestinal pathogenic *E. coli* (ExPEC), which is associated with urinary tract infections (referred to as uropathogenic *E. coli* [UPEC]) and neonatal meningitis (838). However, ExPEC strains rarely share the adhesion, invasion, and intracellular replication traits that identify the AIEC pathotype (839).

Pathoadaptation. Whole-genome sequencing of LF82, the prototypical strain of the AIEC pathotype, and other clinically isolated AIEC strains has aided in the characterization of a number of acquired genes, gene clusters, and pathoadaptive mutations responsible for the AIEC phenotype (837, 840, 841). The organization of the AIEC genome is similar to that of other pathogenic *E. coli* strains, with large regions of the core genome interrupted with genomic islands probably acquired by horizontal transfer (841). The presence of these 35 unique genomic islands likely represents the genetic determinants accounting for the AIEC pathotype contributing to bacterial adherence, invasion, and intracellular survival (837, 841). Genome sequencing has aided the identification of four potential virulence determinants: first, a type VI secretion system, which is utilized by Gram-negative bacteria to export proteins across the cell envelope and is thought to support intracellular survival (837, 841); second, several genes encoding adhesins, specifically type I pili, the associated FimH adhesion tip protein, which is important for adhesion to the intestinal epithelium, and long polar fimbriae that play a crucial role in invasion; third, various transcriptional regulators of virulence genes, which require further study by functional genomics to understand their roles in AIEC survival and growth (837); and finally, genes involved in iron acquisition, which has been shown to be an essential virulence trait in ExPEC strains (837). Although the LF82 strain is considered prototypic for the AIEC pathotype, genome sequencing revealed the presence of an additional plasmid resembling the *Yersinia pestis* pMT1, which is not present in other clinical AIEC isolates (841).

Epidemiology

Several clinical studies have found adhesive and invasive *E. coli* in over 30% of patients with CD (842–845). In a study including 63 CD patients, AIEC was isolated from approximated 36 to 38% of ileal lesions, whereas it was only found in 6% of 102 healthy individuals (843). Further CD patients have increased serum antibody titers raised against *E. coli* (846), especially to outer membrane protein C (OmpC) (847). Increased serum reactivity to OmpC correlates with increased disease severity and can be used as an indicator of CD severity and progression in patients (847). With respect to CD, recent epidemiological studies have shown that incidence and prevalence is rising in all ethnic groups of the developed world; however, the highest incidence is seen in North America, northern Europe, and the United Kingdom. It is estimated that 1.4 million persons in the United States and 2.2 million persons in Europe suffer from this debilitating disease (848). Although epidemiological data on AIEC in connection with CD are limited, the available data suggest a correlation between CD occurrence and the presence of AIEC, making this pathotype a relevant health threat and therapeutic target.

Pathogenesis

The AIEC pathotype is defined by the ability to adhere to and invade epithelial cells and replicate within epithelial cells and macrophages (Fig. 6). Interestingly, many of the virulence strategies used by AIEC are facilitated by gene mutations associated with susceptibility to CD. The initial stage of AIEC pathogenesis in the ileum requires adhesion to host cells. AIEC requires flagellum-mediated motility and utilizes surface expression of type 1 pili to adhere to CAECAM6 expressed on host epithelial cells (849, 850). CAECAM6 expression is increased in CD patients, and this could be due to genetic predisposition or through stimulation of TNF-α and IFN-γ production by AIEC colonization, perpetuating disease pathology (850). After adhesion to epithelial cells, AIEC utilizes several virulence factors to facilitate invasion. One such virulence strategy is the production of OMVs, which deliver bacterial effectors to host epithelial cells (851). OMVs deliver effectors by fusing with epithelial cells through the expression of OmpA, a major membrane-bound protein, which binds the host ER stress response chaperone, Gp96 (852). A subset of CD patients have an SNP in the XBP1 gene locus, causing abnormalities in the ER stress response pathway and increased expression of Gp96 in the ileum (852, 853). The increased expression of Gp96 in CD patients promotes AIEC virulence by enhancing Gp96-mediated invasion. In addition to invasion of intestinal epithelial cells, AIEC is also ca-

pable of translocating through the epithelium to gain access to the underlying lymphoid tissue, the lamina propria. AIEC expresses long polar fimbriae that interact with M cells, a monolayer of specialized epithelial cells at the surface of Peyer's patches, allowing AIEC to access the lamina propria (305). Once AIEC has gained access to the lamina propria, it can infect and replicate within the phagolysosomes of macrophages without inducing macrophage cell death (854, 855). Continuous replication of AIEC in infected macrophages results in secretion of high levels of TNF-α, causing intestinal inflammation and granuloma formation in CD patients (855). CD can be associated with genetic polymorphisms in autophagy-related genes, such as those for ATG16L1 (autophagy-related like 1) and IRGM (immunity-related GTPase family M). Autophagy plays a major role in the prevention of intracellular bacterial replication. Thus, respective genetic polymorphisms can enhance intracellular replication of AIEC in macrophages and epithelial cells, causing heightened inflammation and pathology (856, 857).

Clinical Considerations

Symptoms. AIEC has been implicated in inflammatory bowel diseases in humans and animals, including CD in humans and granulomatous colitis in boxer dogs, chickens, and turkeys (858, 859). Human studies have found that AIEC colonizes the intestinal mucosae of CD patients and is associated with both early and chronic lesions, suggesting that AIEC may initiate disease as well as contribute to chronic inflammation (17, 843, 860). CD presents with abdominal pain, fever, and bowel obstruction or diarrhea with the presence of blood and/or mucus (861).

Detection. In order to identify AIEC strains in intestinal specimens, *E. coli* strains are screened for their ability to invade epithelial cells and to survive and replicate within macrophages using a standard gentamicin protection assay (843).

Treatment. AIEC-mediated CD could be treated utilizing strategies involved in the prevention of AIEC replication, adhesion, and invasion. It has been documented that clinical symptoms of CD improve when luminal bacteria concentrations are reduced via intestinal washes or antibacterial drugs, such as antibiotics (862). Although antibiotics have been used extensively in the clinic to treat CD and other IBDs, continued use should be carefully considered due to their drastic effects on the microbial composition and immune status of the intestine (863). Future therapeutic strategies could involve blocking AIEC adhesion. Based on the recent crystal structure of the interaction between type 1 pili and CAECAM6, it would be feasible to utilize natural or engineered mannose antagonists to block the interaction between FimH and CAECAM6, thereby inhibiting colonization (864, 865). Adhesin-based vaccines could also be an effective strategy to block AIEC colonization in the gut, as these vaccines have shown success in blocking UPEC colonization in the mouse bladder (866). The function of type 1 pili, and hence ability of AIEC to colonize the ileum, could also be inhibited by preventing pilus assembly using currently available pilicides (867). Adhesion of AIEC could be competitively inhibited through administration of yeast-based probiotics, as they express cell walls that are rich in free mannose residues, which would act as decoy ligands for type 1 pili (865). Lastly, therapeutic strategies could be developed that target the host ER stress response chaperone, Gp96, or OMV surface-expressed proteins, specifically OmpA, in order to block AIEC invasion of intestinal epithelial cells (852).

CONCLUSIONS

The diversity of *E. coli* strains is remarkable, ranging from harmless inhabitants of the gastrointestinal tract to diverse pathogens capable of causing intestinal or extraintestinal diseases. While some clinical outcomes are more severe than others, *E. coli* still remains a major public health concern. It is clear that pathogenic *E. coli* continues to evolve, as exemplified by the EAEC and STEC hybrid strain that caused a large outbreak throughout Europe in 2011. This also points to the ease of transmissibility of pathogenic *E. coli*; the low infectious doses of many of the pathotypes and the potential to disseminate among a variety of sources are extraordinary. Food, water, companion pets, animals, and other people are all potential points of contamination and transmission.

New technologies are needed to rapidly identify, characterize, and monitor pathogenic *E. coli*. It is hoped that the development of rapid pathogen identification strategies will provide clinicians faster diagnosis for quick and appropriate illness management for patients. The depth and wealth of information that can be acquired through high-throughput sequencing will help inform clinical needs (e.g., potential antibiotic resistance and identification of pathogen-specific virulence factors) and epidemiology and help monitor spread of pathogens, as has been nicely reviewed elsewhere (868). An excellent example of the possibility and utility of rapid sequencing occurred during the German STEC O104:H4 outbreak (396), and this was further exemplified by the assembly of a draft genome of STEC O104:H4 using high-throughput sequencing during a retrospective study of stool samples (869). Whole-genome sequences can also provide high-resolution detail for typing and outbreak investigations, as it has been shown that analysis of genome sequences can provide high-resolution typing of outbreak strains (870). The limiting factor for all this information, however, is still meaningful and reliable bioinformatics. While it is very possible to sequence and generate a draft genome of a given pathogen in a day or two, quality assurance is needed for the nucleic acid information. In addition, there are still too many hypothetical genes and genes of unknown function, which hinders our ability to fully understand these pathogens. By generating more genomic data sets, we will undoubtedly uncover more genes (i.e., contributing to the pangenome). The amount of information derived from whole-genome sequences will provide a deeper understanding of the relationships between pathogens and their hosts, how they evolve, and how this ultimately affects human health.

ACKNOWLEDGMENTS

Work in the laboratory of B.B.F. is supported by the Canadian Institutes of Health Research (CIHR) (funding reference no. MOP-13452). B.B.F. is the University of British Columbia Peter Wall Distinguished Professor. R.J.L is supported by a Natural Sciences and Engineering Research Council of Canada (NSERC) postgraduate scholarship. K.M.K. is supported by the National Institutes of Health under Ruth L. Kirschstein National Research Service Award FAIO85761A. M.W. is supported by a CIHR Frederick Banting and Charles Best Canada graduate scholarship.

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Roland Scholz received his training in biochemistry and microbiology at the University of Regensburg, Regensburg, Germany, the University of Colorado at Boulder, Boulder, CO, USA, and the Max Planck Institute of Biochemistry, Martinsried, Germany. He obtained his Ph.D. training at the Institute of Cell Biology, ETH Zurich, Zurich, Switzerland. Currently he is a postdoctoral trainee in the laboratory of Professor B. Brett Finlay at the University of British Columbia, Vancouver, Canada. With more than 12 years of experience in the fields of biochemistry, cell biology, and microbiology, Dr. Scholz currently studies the molecular mechanisms of host-pathogen interactions, with a focus on the human pathogens enteropathogenic and enterohemorrhagic *Escherichia coli.* By unraveling the complex molecular interplay between host and pathogen, he aims to provide the basis for novel therapeutic approaches. Dr. Scholz is an author of 13 peer-reviewed scientific publications.

Continued next page

Kristie Keeney has more than 12 years of experience in microbiology and immunology and has eight peer-reviewed scientific publications. Dr. Keeney is a senior postdoctoral trainee in the laboratory of Professor B. Brett Finlay, a world leader in the field of microbiology and bacterial pathogenesis, at the University of British Columbia, Vancouver, Canada. She obtained her B.A. in biochemistry and molecular biology at Reed College in Portland, OR, and her Ph.D. in microbiology and immunology at the University of Michigan, Ann Arbor. In her postdoctoral study, Dr. Keeney is studying the human pathogen enteropathogenic *Escherichia coli* by using *Citrobacter rodentium* in a mouse model of infection and is identifying new targets for drug design and probiotic treatment for the prevention of pathogenic colonization. Dr. Keeney has won several awards and is currently a Ruth L. Kirschstein National Research Service Award (NRSA) postdoctoral fellow.

Marta Wlodarska is currently a senior Ph.D. candidate in the laboratory of Professor B. Brett Finlay. She has received several prestigious awards for her doctoral work, including the Canada Graduate Scholarship from the Canadian Institutes of Health Research, a Michael Smith Foundation for Health Research and Crohns & Colitis Foundation Trainee Award, and the Pacific Century Graduate Award and Four Year Fellowship Award from the University of British Columbia. She obtained her B.Sc. also at the University of British Columbia with honors in microbiology and immunology. Currently her research focuses on understanding intestinal mucin dynamics and their implications for gastrointestinal health.

B. Brett Finlay is a Professor in the Michael Smith Laboratories and the Departments of Biochemistry and Molecular Biology and of Microbiology and Immunology at the University of British Columbia. He obtained a B.Sc. (Honors) in biochemistry at the University of Alberta, where he also did his Ph.D. in biochemistry under Dr. William Paranchych. His postdoctoral studies were performed with Dr. Stanley Falkow at the Department of Medical Microbiology and Immunology at Stanford University School of Medicine. In 1989, he joined the University of British Columbia as an Assistant Professor in the Biotechnology Laboratory. Dr. Finlay's research interests are focused on host-pathogen interactions at the molecular level. His laboratory studies several pathogenic bacteria, where interactions of *Salmonella* and pathogenic *E. coli* with host cells are the primary focus. Additionally, he is interested in the role of microbiota in enteric and allergic diseases.